Inhibition of Acrylamide Generation by Hydrostatic Pressure and Cysteine Addition

Atsushi KOBAYASHI1,2,†, Satoko GOMIKAWA2, Asami OGURO2, Akira YAMAZAKI2, Shinji SATO3, Hirofumi MAEKAWA1

1Nagaoka University of Technology, Kamitomioka-machi, Nagaoka, Niigata 940–2188, Japan.
2Research Institute, Echigoseika Co., Ltd., Takanashi-machi, Ojiya, Niigata 947–0193, Japan.
3Laboratory of Functional and Analytical Food Science, Niigata University of Pharmacy and Applied Life Science, Higashijima, Akiha-Ku, Niigata 956–8603, Japan.

We investigated the effect of medium high hydrostatic pressure (<100 MPa) on acrylamide generation and the Maillard reaction using an equimolar asparagine–glucose aqueous solution adjusted to pH 9.0. The amounts of acrylamide and melanoids generated and pH were determined after treatment at 70℃ and 60 or 90 MPa pressure or atmospheric pressure for up to 72 hours. Comparison of acrylamide and melanoids contents revealed that pressure inhibited acrylamide generation in this solution. When added to the same solution, cysteine markedly inhibited acrylamide generation independent of pressure under the condition of 70℃ at 90 MPa or atmospheric pressure for 24 hours. Based on these results, under the same condition as the cysteine addition experiment, we examined the inhibitory effect of pressure and cysteine on acrylamide generation using a non–centrifugal cane sugar (NCS) solution at pH 5.5, which contains a relatively large amount of acrylamide. Adding cysteine to the NCS solution reduced its original amount of acrylamide and inhibited acrylamide generation during heat treatment. Pressurization of a cysteine-free NCS solution promoted acrylamide generation, but that of an NCS solution with high cysteine concentration promoted acrylamide decrease. These results suggest that acrylamide generation and the Maillard reaction during food processing might be regulated by medium high hydrostatic pressure and cysteine addition.

Keywords: acrylamide, Maillard reaction, hydrostatic pressure, non–centrifugal cane sugar, cysteine

1. Introduction

Acrylamide, a potential human carcinogen [1], is produced in carbohydrate–rich foods such as potatoes when processed at temperatures above 120℃ [2]. Acrylamide in processed foods is generated through the Maillard reaction during high-temperature cooking and is thought to be primarily formed from asparagine and reducing sugars, such as glucose and fructose (Fig. 1). The Maillard reaction plays an important role in food processing to improve flavor and color [3,4]. As it is important to suppress acrylamide formation in processed foods while maintaining high quality, the food-processing industry requires the development of a method to control the Maillard reaction.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA), the international organization of risk assessment of food contaminants, considers acrylamide in foods to be a human health concern and recommends further efforts to mitigate acrylamide formation in foods [5,6]. In Japan, the Ministry of Agriculture, Forestry and Fisheries (MAFF) published a “guideline for reducing acrylamide in foods” for food-related businesses [i] as well as results from large-scale surveys on the acrylamide content of foods on the market from 2003 to 2016 [ii–v]. In these surveys, acrylamide was detected in rice crackers, a traditional Japanese snack. Rice cracker dough contains reducing sugars and amino acids and is processed at high temperatures, namely baking or frying; therefore, rice crackers would contain acrylamide. Moreover, to fulfill consumer needs, the manufacturing of rice crackers uses a variety of flavor materials and processing methods. In the present study, we surveyed the acrylamide content of rice crackers and others in the market. As shown in Table 1, 10 of 14 rice crackers con-
Atsushi KOBAYASHI, Satoko GOMIKAWA, Asami OGURO, Akira YAMAZAKI, Shinji SATO, Hirofumi MAEKAWA

Fig. 1 Formation of acrylamide from asparagine in the presence of reducing sugars.

Acrylamide content of rice crackers is mainly affected by flavor materials like NCS, which agrees with the large-scale survey data published by MAFF. Lowering the concentration of acrylamide in the NCS used in rice crackers will decrease acrylamide in the final products.

High hydrostatic pressure (HHP) treatment is a food-processing technique that employs hydrostatic pressure with a range of tens to hundreds of megapascals (MPa). We previously reported that physical denaturation, fortification of functional compounds, and pasteurization of foods are achieved with HHP treatment at 400 MPa or less. In food processing, HHP treatment for a few to ten minutes has generally been used for food materials or intermediate and final products, in combination with heat treatment if necessary. In addition, the fermentation and aging of foods by degrading enzymes can be promoted by HHP treatment ≤100 MPa for hours to days while suppressing the growth of microorganisms.

We previously reported the inhibitory effect of HHP (100 to 300 MPa, 120°C) on acrylamide formation using an equimolar solution of asparagine and glucose. Progression of the Maillard reaction under hydrostatic pressure has been reported; on the other hand, there have been few reports on the formation of acrylamide. Moreover, the effect of medium high hydrostatic pressure (<100 MPa) on toxic substance formation, including acrylamide, has not yet been reported. The increase of temperature always accelerates reaction rates, whereas the increase of pressure accelerates or retards reaction rates. The application of pressure combined with heating would increase the yield of profitable products or decrease that of unwanted products.

This study aims to inhibit the generation of acrylamide in processed food by medium high hydrostatic pressure treatment (<100 MPa). At first, we determined the acrylamide concentration after treatment at 70°C at a pressure of 60 or 90 MPa or atmospheric pressure for up to 72 hours using an equimolar asparagine-glucose aqueous solution adjusted to pH 9.0. We also determined the melanoidins concentration and pH of the treated solution as indicators of the degree of Maillard reaction progression, and then evaluated the inhibitory effect of pressure on acrylamide generation and progression of the Maillard reaction. Also, we previously reported that adding cysteine to NCS, which contains considerable amounts of asparagine and reducing sugars, inhibits acrylamide generation during heating. Therefore, the same analyses as above were performed after treatment at 70°C at a pressure of 90 MPa or atmospheric pressure for 24 hours.
Pressure and Cys inhibit acrylamide generation

2. Materials and Methods

2.1 Materials

Acrylamide (ultra-pure, >99.9%) was purchased from Kanto Chemical (Tokyo, Japan) and \(^{13}\)C-labelled acrylamide (>98%), for use as an internal standard (IS), was from CDN Isotopes (Montreal, QC, Canada). Analytical grade L-asparagine monohydrate (99.0%), D- (+)-glucose (98.0%), N-tris (hydroxymethyl) methyl-3-amino-1-propanesulfonic acid (TAPS buffer, 99.0%), and all other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Potassium hexacyanoferrate (II) trihydrate solution [15% (wt/vol)] and zinc sulfate heptahydrate solution [30% (wt/vol)] were prepared as Carrez reagents I and II. An NCS (kokuto) sample made in Japan was obtained on the consumer market.

2.2 Sample preparation

The heating experiment under hydrostatic pressure was performed using an aqueous model system prepared by mixing equimolar concentrations (0.125 mol/L) of asparagine and glucose in 0.1 mol/L TAPS buffer, as previously reported [16]. The initial pH of this model system was adjusted to 9.0 with 0.1 mol/L NaOH. A similar experiment was performed using the same model system containing 0.0125 mol/L L-cysteine.

Table 1  Acrylamide content in rice crackers and other foods.

| No. | Sample                                      | Acrylamide content (μg/kg) |
|-----|---------------------------------------------|----------------------------|
| 1   | Rice cracker 1 (senbei, baked)              | 35.9±4.8                   |
| 2   | Rice cracker 2 (senbei, baked, soy sauce)   | <25                        |
| 3   | Rice cracker 3 (senbei, baked, soy sauce)   | <25                        |
| 4   | Rice cracker 4 (okaki, baked, salt)         | 204.5±8.3                  |
| 5   | Rice cracker 5 (okaki, baked, sweet)        | 2351.6±43.2                |
| 6   | Rice cracker 6 (okaki, fried, soy sauce)    | <25                        |
| 7   | Rice cracker 7 (okaki, baked, sweet)        | <25                        |
| 8   | Rice cracker 8 (okaki, baked, cheese)       | <25                        |
| 9   | Rice cracker 9 (senbei, baked)              | 36.9±1.7                   |
| 10  | Rice cracker 10 (okaki, baked)              | 28.9±2.0                   |
| 11  | Rice cracker 11 (okaki, baked, mixed with sugar) | <15                      |
| 12  | Rice cracker 12 (okaki, baked, mixed with cheese) | 299.8±6.9               |
| 13  | Rice cracker 13 (okaki, baked, mixed with soybean) | 306.9±16.8            |
| 14  | Rice cracker 14 (senbei, extruded, mixed with cheese) | 49.6±3.8               |
| 15  | Japanese deep-fried cookie (karinto)        | 1321.0±8.7                 |
| 16  | Brown sugar                                 | 474.4                      |
| 17  | Fried potato 1                              | 419.0±65.4                 |
| 18  | Fried potato 2                              | 303.4±60.0                 |
| 19  | Fried potato 3                              | 277.1±44.7                 |
| 20  | Instant coffee                              | 129.1±33.4                 |
| 21  | Soy sauce                                   | trace                      |
| 22  | Fried kelp                                  | 26.3±4.3                   |
| 23  | Roasted soybean powder                      | 54.4±1.8                   |
| 24  | Wasanbon                                    | 50.6±7.2                   |
| 25  | NCS 1                                       | 478.5±9.7                  |
| 26  | NCS 2                                       | 164.4±9.8                  |
| 27  | NCS 3                                       | 303.1±2.0                  |
| 28  | NCS 4                                       | 128.3±1.9                  |

Mean±SD (n=3; for brown sugar only, n=1)

Acrylamide content in Nos. 1-8 and Nos. 15-20 was determined by LC/MS (LOD=15, LOQ=25 μg/kg) and that in Nos. 9-14 and Nos. 21-28 was determined by LC-MS/MS (LOD=5, LOQ=15 μg/kg). NCS samples (Nos. 25-28) are referenced from Kobayashi et al. [22].

using an asparagine–glucose solution containing cysteine. Furthermore, the amount of acrylamide generated, the degree of browning, and pH of the NCS solutions were determined after the same treatment as the cysteine addition experiment to examine the effect of pressure and cysteine on acrylamide generation.
The heat–pressure treatment of the NCS solution sample was performed under the same conditions as the preceding experiment. The NCS sample was milled in a mortar, and a 12-g aliquot was placed in a glass beaker along with three mL of 20 or 100 mmol/L cysteine and 9 mL of water. The NCS in the beaker was dissolved with a glass stirring stick, and the resulting solutions were used as the cysteine–containing NCS samples. The final molar amounts of cysteine in the samples were 0.06 and 0.3 mmol, respectively. A control and a cysteine–free NCS sample were prepared as above, except that the cysteine solution was replaced with water.

2.3 Pressurization equipment and processing

Figure 2 shows a schematic of the pressurization equipment. The set-up consists of a custom-build pressure vessel (158 mm outer diameter, 100 mm internal diameter, 428 mm height; Echigoseika, Niigata, Japan) equipped with an internal temperature sensor (SCHS1-0 KT128G637, Chino Corporation, Tokyo, Japan), a pressure sensor (PG-2TH, Kyowa Electronic Instruments, Tokyo, Japan), and binary hand pumps, i.e., a manual pressurization pump and a pressure control pump (P-1B-S2, Riken Seiki, Niigata, Japan). This equipment displays temperature and pressure data detected by the sensors on a monitor (Omron, Kyoto, Japan). The equipment is rated to a maximum pressure of 100 MPa; this experiment applied pressures at 60 and 90 MPa. The heating medium was sent from a low-temperature bath (NCB-2600, Tokyo Rikakikai, Tokyo, Japan) and heated the pressure vessel by flowing around it. The pressure vessel and the low-temperature bath were filled with an antifreeze solution (NYBRINE Z1, MORESCO Corporation, Hyogo, Japan) used as the pressure and heating medium.

A 10-mL glass screw vial (22.5 mm outer diameter, 64 mm height, for headspace gas chromatography, GL Science, Tokyo, Japan) was filled with the sample solution and sealed with a steel cap (18 mm capsize, 8 mm hole size, including a PTFE/silicone septum, GL Science) while removing as much air as possible. The vial was placed in a plastic bag with 20 mL of hot water (72°C), which was put in the pressure vessel preheated at 70°C. The pressure vessel was instantly closed and then pressurized with the manual pressurization pump. The reaction period was started after the vessel reached the desired pressure level (60 or 90 MPa). The reaction periods of heating experiments under pressure were 16, 24, 48, or 72 hours. The experiments involving cysteine augmented samples, and NCS samples were conducted for 24 hours. By handling the pressure control pump during the reaction, the pressure in the pressure vessel was held at the desirable level. After the reaction, the pressure in the pressure vessel was released by opening the drain valve. Removing the sample bag from the pressure vessel was immediately followed by cooling it in ice water to stop the reaction from proceeding. After cooling, the sample vial was pulled out from the bag and warmed to room temperature. Control experiments were carried out using the

---

Fig. 2 Schematic diagram of the pressure reaction system manufactured by Echigoseika Co., Ltd.
above method under atmospheric pressure in a water bath at 70°C. Browning and the pH of the samples were determined, and acrylamide was extracted and purified from the samples.

2.4 Acrylamide extraction

Acrylamide was extracted and purified as described previously [16]. A 1–mL aliquot of the asparagine and glucose solution sample diluted to the proper concentration, a 1–g aliquot of the NCS solution sample, or a 2 g of the control NCS solution sample was pipetted into a 30–mL polypropylene high-speed centrifuge tube. Water (9 mL) and $^{13}$C$_1$-acrylamide (10 μL of 100 μg/mL) were added to the sample prior to being shaken vigorously. The mixture was treated with Carrez reagents (1 mL of reagent I and 1 mL of reagent II) with stirring and centrifuged at 15,000×g and 10°C for 20 min (RS-18IV, Tomy Seiko, Tokyo, Japan). A 1.5 g of sodium chloride was added to the supernatant (5 mL) in a 30–mL glass centrifuge tube and completely dissolved by shaking the tube. Ten milliliters of ethyl acetate was added to the solution, and the tube was shaken vigorously for 1 min. The organic phase was collected in a 100–mL polypropylene high-speed centrifuge tube. Water loaded onto a preconditioned 500 mg-Isolute Multimode cartridge (3 mL of methanol and 6 mL of water, International Sorbet Technology, Glamorgan, UK), eluted, and collected. The cartridge was rinsed with 1 mL of water, and the eluate was collected and combined with the previous eluate in a 10–mL glass vial. The combined eluate was concentrated to 500 μL by blowing nitrogen gas while heating at 40°C. The condensed eluate was filtered through a 0.20–μm pore-sized filter unit and stored at −18°C until liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis.

2.5 Acrylamide quantification by LC–MS/MS

Acrylamide was analyzed on an LCMS–8030A (Shimadzu, Kyoto, Japan) as described previously [16]. The analytical separation was performed on a reverse-phase C18 column (Synergi Hydro–RP, 250 mm×2 mm i.d., 4 μm, Phenomenex, CA, USA) using the isocratic mixture of 0.1 vol% acetic acid and methanol (98:2, v/v) at a flow rate of 0.2 mL/min at 40°C. The sample injection volume was 10 μL. The ionization mode was set to positive electrospray ionization (ESI+). Multiple reaction monitoring (MRM) mode was applied to detect and identify acrylamide (m/z 72.10–54.95) and IS (m/z 73.10–55.95). An acrylamide calibration curve was obtained from the peak area ratio of acrylamide (0–1000 ppb) to IS (1000 ppb). The detection and quantification limits of acrylamide were 5 ppb and 15 ppb, respectively.

2.6 Determination of browning

Browning compounds were quantified by measuring absorbance at 470 nm using an ultraviolet-visible spectrophotometer (UV mini-1240, Shimadzu), as described previously [16]. Samples were analyzed following dilution to an appropriate concentration, as needed. The corresponding melanoids concentrations, formed from asparagine and glucose, could be calculated from the Lambert–Beer equation using the measured absorbance and an extinction coefficient of 282 L/mol·cm, as described by Knol et al. [23].

Browning of NCS solution samples was determined using the method described by Maeda et al. [24]. NCS solution samples were diluted 10 times with water and centrifuged at 1,100×g for 15 min, and the absorbance of the supernatant was measured at 470 nm.

2.7 pH measurement

Treated sample pH was measured with a pH meter (Horiba, Kyoto, Japan) equipped with a glass electrode at room temperature.

2.8 Statistical analysis

All experiments were conducted in triplicate. Experimental results are expressed as means±standard deviation. Statistical analysis of each experimental reaction time and comparison with controls in the NCS experiments were conducted using Student’s t-test. Correlations among the factors at each reaction time were assessed by determining the Pearson correlation coefficients. The cysteine–added experiments were statistically analyzed using a two–way ANOVA; Tukey’s multiple comparison test was applied where significant interactions between pressure and cysteine addition were observed. Statistically significant differences were defined at $p<0.05$ or $p<0.01$. 

© 2021 Japan Society for Food Engineering
3. Results

3.1 The effect of pressure on acrylamide generation, melanoidins generation, and pH in the asparagine-glucose model system

Figure 3A shows the amount of acrylamide in asparagine-glucose solutions heated to 70°C for 16, 24, 48, and 72 hours under hydrostatic pressure up to 90 MPa. The amount of acrylamide showed a time-dependent increase under all experimental pressure conditions. The amounts of acrylamide generated under heat-pressure reactions lasting 16, 24, 48, and 72 hours were 0.69–0.84, 1.42–1.69, 3.32–3.80, and 4.78–5.75 ppm, respectively. In comparison with samples treated at atmospheric pressure (0.1 MPa), samples treated at 60 MPa contained high amounts of acrylamide following reaction times of 16 and 24 hours (both \( p < 0.01 \)) and had a relatively low amount at 72 hours. In contrast, the amounts of acrylamide in samples treated at 90 MPa were not significantly different from those at 0.1 MPa at reaction times of 16 and 24 hours, and were lower than amounts at 0.1 MPa at 48 and 72 hours (\( p < 0.05 \) and <0.01).

Table 2 shows absorbance at 470 nm in the asparagine-glucose solution after the above-described treatments. The amounts of melanoidins were calculated from Table 2 values, shown in Fig. 3B. The amount of melanoidins increased with reaction time and pressure. The amounts of melanoidins generated under the heat-pressure reaction lasting 16, 24, 48, and 72 hours were 0.03–0.06, 0.07–0.18, 0.38–0.77, and 0.76–1.46 mmol/L, respectively. Samples treated at 60 and 90 MPa contained higher amounts of melanoidins than those treated at 0.1 MPa at all experimental reaction times (all \( p < 0.01 \)). In particular, the amount of melanoidins in all 90 MPa samples were about twice that observed in the 0.1 MPa samples, indicating that pressure promotes the Maillard reaction.

Table 2   Absorbance at 470 nm in the asparagine-glucose model system in TAPS buffer (initial pH 9.0) heated at 70°C for 24 hours.

| Time (h) | 0.1 MPa | 60 MPa  | 90 MPa   |
|---------|---------|---------|----------|
| 0       | 0       | 0       | 0        |
| 16      | 0.074±0.021 | 0.147±0.003 | 0.172±0.002 |
| 24      | 0.204±0.019 | 0.450±0.010 | 0.504±0.013 |
| 48      | 1.062±0.045 | 1.912±0.061 | 2.174±0.066 |
| 72      | 2.134±0.009 | 3.598±0.144 | 4.128±0.078 |

Mean±SD (\( n = 3 \))
Pressure and Cys inhibit acrylamide generation

Figure 3C shows the pH of an asparagine–glucose solution after the above-described treatments. The pH of samples treated at 60 MPa for 24 hours and at 90 MPa for 16 hours was reduced compared with those at 0.1 MPa for 24 and 16 hours, respectively ($p<0.01$ and $<0.05$). The pH of samples treated at 60 and 90 MPa for 48 hours decreased substantially compared with those at 0.1 MPa for 48 hours (both $p<0.01$). Treatment of samples at 60 and 90 MPa for 72 hours resulted in lower pH than values observed at 0.1 MPa, however, the pH values were higher than corresponding samples treated for 48 hours.

Figure 4 shows the pressure dependence of the reaction rate constants ($k$) for the generation of acrylamide and melanoidins. The reaction rate constants for each product were calculated from the amounts produced per reaction time, all of which were observed as zero-order reactions. The activation volumes ($\Delta V^\neq$) of the formation reactions of each component were calculated from the slope of a line plotting $\ln k$ against pressure. The $\Delta V^\neq$ values determined were +5.4 mL/mol and -21.6 mL/mol for acrylamide and melanoidins, respectively.

### 3.2 Correlations among pressure, acrylamide generation, melanoidins generation, and pH in an asparagine–glucose solution

Table 3 shows the correlations among pressure, acrylamide concentration, melanoidins concentration, and pH change at each reaction duration. For all reaction durations, pressure was positively and strongly correlated with melanoidins concentration (16, 24, 48, and 72 hours: $r=0.969, 0.982, 0.989$, and $0.993$). In addition, the correlations between pressure and acrylamide concentration, and between acrylamide and melanoidins concentration, changed from weakly positive to negative with increasing reaction duration, finally reaching significance at 72 hours (pressure vs. acrylamide: $r=0.387, 0.130, -0.494$, and $-0.899$, acrylamide vs. melanoidins: $r=0.453, 0.289, -0.369$, and $-0.845$). The strong negative correlations between pressure and pH change and between melanoids concentration and pH change were significant except for at 24 hours (pressure vs. pH: $r=-0.803, -0.384, -0.985$, and $-0.891$, melanoidins vs. pH: $r=-0.717, -0.523, -0.995$, and $-0.927$).

### 3.3 The effect of cysteine and pressure on acrylamide generation, melanoidins generation, and pH in the asparagine–glucose model system

Figure 5A shows the amount of acrylamide in cysteine-containing or cysteine-free asparagine–glucose solutions heated at 70°C for 24 hours under hydrostatic pressures of 0.1 and 90 MPa. The amount of acrylamide was reduced by approximately 80% in the cysteine-containing sample solution, indicating that cysteine had a high inhibitory effect on acrylamide generation (two-way ANOVA, $p<0.01$). In contrast, an applied pressure of 90 MPa had no significant effect on acrylamide generation, and there was no interaction between pressure and cysteine addition.

Table 4 shows absorbance at 470 nm in the cysteine-containing asparagine–glucose solutions after the above-described treatments. The amounts of melanoidins were calculated from Table 4 values, shown in Fig. 5B. Both pressure and cysteine addition affected the generation of melanoidins in the sample solution (two-way ANOVA, pressure: $p<0.01$, cysteine: $p<0.05$). In addition, there was a significant interaction between pressure and cysteine addition ($p<0.01$). Based on this result, Tukey’s multiple comparisons of all groups showed that adding cysteine to the sample solution increased melanoidins generation at 0.1 MPa, but inhibited melanoidins generation at 90 MPa. This indicates that cysteine inhibited the effect of pressure on melanoidins generation.

Figure 5C shows the pH of cysteine-containing or cysteine-free asparagine–glucose solutions after the above-described treatments. Both pressure and cysteine addition affected pH changes in the sample solutions, with significant interactions observed between the factors (two-way ANOVA, all $p<0.01$). Analysis with Tukey’s multiple comparison test of all groups confirmed that
### Table 3  Correlation coefficient matrices for each time point.

#### Reaction time of 16 hours

|                      | Pressure (MPa) | Acrylamide (ppm) | Melanoidins (mmol/L) | pH           |
|----------------------|----------------|-------------------|----------------------|--------------|
| Pressure (MPa)       | 1              | 0.387             | 0.969*               | -0.803*      |
| Acrylamide (ppm)     |                | 1                 | 0.453                | -0.474       |
| Melanoidins (mmol/L) |                |                   | 1                    | -0.717*      |
| pH                   |                |                   |                      | 1            |

#### Reaction time of 24 hours

|                      | Pressure (MPa) | Acrylamide (ppm) | Melanoidins (mmol/L) | pH           |
|----------------------|----------------|-------------------|----------------------|--------------|
| Pressure (MPa)       | 1              | 0.130             | 0.982*               | -0.384       |
| Acrylamide (ppm)     |                | 1                 | 0.289                | -0.857*      |
| Melanoidins (mmol/L) |                |                   | 1                    | -0.523       |
| pH                   |                |                   |                      | 1            |

#### Reaction time of 48 hours

|                      | Pressure (MPa) | Acrylamide (ppm) | Melanoidins (mmol/L) | pH           |
|----------------------|----------------|-------------------|----------------------|--------------|
| Pressure (MPa)       | 1              | -0.494            | 0.989*               | -0.985*      |
| Acrylamide (ppm)     |                | 1                 | -0.369               | 0.359        |
| Melanoidins (mmol/L) |                |                   | 1                    | -0.995*      |
| pH                   |                |                   |                      | 1            |

#### Reaction time of 72 hours

|                      | Pressure (MPa) | Acrylamide (ppm) | Melanoidins (mmol/L) | pH           |
|----------------------|----------------|-------------------|----------------------|--------------|
| Pressure (MPa)       | 1              | -0.899*           | 0.993*               | -0.891*      |
| Acrylamide (ppm)     |                | 1                 | -0.845*              | 0.619        |
| Melanoidins (mmol/L) |                |                   | 1                    | -0.927*      |
| pH                   |                |                   |                      | 1            |

*Significant correlation at the 0.05 level.

![Fig. 5](image)

**Fig. 5** The effect of cysteine and pressure on (A) acrylamide generation, (B) melanoidins generation, and (C) pH in the asparagine-glucose model system in TAPS buffer (initial pH 9.0) heated at 70°C for 24 hours. Experimental results are expressed as means±SD (n=3). Data were statistically analyzed by a two-way ANOVA, and Tukey’s multiple comparison test was applied where significant interactions between pressure and cysteine addition were observed. Different letters indicate significant differences between groups (p<0.05). Final concentrations of cysteine were as follows; Cys (−)=0 M, Cys (+)=0.0125 M.
adding cysteine to the sample solution resulted in higher pH values than in the cysteine-free sample, irrespective of pressurization. In the cysteine-containing sample, less pH change was observed at 90 MPa relative to 0.1 MPa.

### 3.4 The effect of cysteine and pressure on acrylamide generation, browning, and pH in NCS solutions

Figure 6A shows the amount of acrylamide in cysteine–containing or cysteine–free NCS solutions heated to 70°C for 24 hours under hydrostatic pressures of 0.1 and 90 MPa. The control solution (untreated NCS solution) contained 0.143 ppm of acrylamide. Compared with the control, the cysteine–free NCS solution and the NCS solution with a low cysteine concentration contained increased amounts of acrylamide (Student’s t-test, \( p < 0.01 \)). Moreover, the NCS solution with a low cysteine concentration exhibited reduced acrylamide generation compared with the cysteine–free NCS solution. In contrast, the NCS solution with a high cysteine concentration contained lower acrylamide amounts than the control (\( p < 0.01 \)). These results indicate that in NCS, cysteine decreases acrylamide and inhibits acrylamide generation. Additionally, pressure and cysteine addition have effects on acrylamide amount in NCS solutions, with a significant interaction observed between pressure and cysteine addition (two-way ANOVA except for control, \( p < 0.01 \)). Under both pressure conditions, increases in acrylamide amounts were inhibited with increasing cysteine (Tukey’s multiple comparisons, \( p < 0.05 \)). On the other hand, the presence of cysteine changed the effect of pressure on acrylamide. Pressure promoted acrylamide generation in the cysteine–free NCS solution (\( p < 0.05 \)); pressure had no effect on acrylamide in the NCS solution with a low cysteine concentration (\( p > 0.05 \)); pressure promoted decreased acrylamide amounts in the NCS solution with a high cysteine concentration (\( p < 0.05 \)).

Figure 6B shows absorbance at 470 nm in cysteine–containing or cysteine–free NCS solutions after the above–described treatments. Statistical analysis of absorbance measurements was performed on the assumption that a certain amount of variation would occur.
interaction between the factors was observed (two-way ANOVA except for control, Student’s t-test, $p < 0.05$). Pressure and cysteine addition had effects on browning degree in NCS solutions, and a significant interaction between the factors was observed (two-way ANOVA except for control, Student’s t-test, $p < 0.01$). Under atmospheric pressure (0.1 MPa), the degree of browning in the NCS solution decreased with increasing cysteine (Tukey’s multiple comparison, $p < 0.05$). At 90 MPa, the degree of browning in the NCS solution with a high cysteine concentration was lower than in the cysteine–free NCS solution and the NCS solution with a low cysteine concentration (90 MPa, Cys (−) vs. Cys (high), $p < 0.05$; Cys (low) vs. Cys (high), $p < 0.05$; Cys (−) vs. Cys (low), $p > 0.05$). Pressure inhibited browning in the cysteine–free NCS solution and the NCS solution with a high cysteine concentration ($p < 0.05$). In contrast, pressure promoted browning in the NCS solution with a low cysteine concentration ($p < 0.05$).

Figure 6C shows the pH of cysteine–containing or cysteine–free NCS solutions after the above-described treatment. The pH of the control solution was 5.22, and the pH of all samples decreased after treatment compared with the control (Student’s t-test, $p < 0.01$). The NCS solution with a high cysteine concentration treated at 90 MPa exhibited the lowest pH. Pressure and cysteine addition decreased pH in the treated NCS solutions, and a significant interaction was observed between pressure and cysteine addition (two-way ANOVA except for control, $p < 0.01$). Under both pressure conditions, pH of the NCS solutions decreased with increasing cysteine (Tukey’s multiple comparison, $p < 0.05$). Pressure decreased the pH of the NCS solution with a high cysteine concentration ($p < 0.05$).

4. Discussion

The inhibitory effect of pressure on acrylamide generation was investigated at pressures < 100 MPa using an aqueous solution model of an equimolar asparagine–glucose mixture or NCS. Pressure experiments were performed with mild heating at 70°C for up to 72 hours.

Pressure treatment (≤90 MPa) of the asparagine–glucose mixture solution inhibited acrylamide generation but promoted melanoidins generation at pH 9.0 (Fig. 3A and B). Hill et al. [18] reported that pressure does not lead to other reactions producing different compounds, as there is no difference in the mechanism with or without elevated pressure for Maillard browning. Ames [17] and Hill et al. [18] also reported pressure-temperature experiments (600 MPa, 50°C) using a glucose–lysine solution and noted that the pressure effect on the rate of Maillard browning varies with solution pH. At an initial pH of 5.1 and 6.5, the rate of Maillard browning was retarded by pressure, but at an initial pH of 8.0 and 10.1, the reaction was significantly enhanced [18]. Because the effect of an initial pH of 7.0–7.5 was negligible, the effect of pressure seemed to be reversed in the pH region of 7.0–7.5 [17,18]. Moreno and coworkers [25] reported a similar study in which the different stages of the Maillard reaction are affected by high pressure (400 MPa) in distinct ways. They noted that in unbuffered and buffered media at an initial pH of 10.2, high pressure promoted the formation and subsequent degradation of Amadori rearrangement products, leading to increased intermediate and advanced reaction products [25].

In Table 3, the correlation of pressure with melanoidins generation was invariably stronger than that of pressure with acrylamide generation at all time points. From these results, we infer that pressure < 90 MPa has a low impact on acrylamide formation reactions but a high impact on the intermediate and advanced stages in the Maillard reaction leading to melanoidins formation. As shown in Fig. 1, acrylamide and melanoidins are formed through a common reaction pathway involving Schiff base formation; Amadori rearrangement from the Schiff base leads to the intermediate stage of the Maillard reaction; and decarboxylation from the Schiff base leads to acrylamide formation. Thus, we infer that pressure promotes melanoidins generation due to acceleration of the conversion from the Schiff base to the Amadori rearrangement product and slightly inhibits acrylamide generation via the concurrent decrease in the amount of decarboxylated Schiff bases. It might also be concluded that pressure promotes the degradation and/or polymerization of the formed acrylamide.

Besides, in Table 3, the negative correlations between pressure and acrylamide generation and between acrylamide and melanoidins generation gradually strengthened over time. These correlations indicate that the inhibitory effect of pressure on acrylamide generation became pronounced as the reaction time increases, i.e., as the Maillard reaction progresses.

In regards to pH, the pH of samples treated under pressure tended to be lower than that under atmospheric pressure (Fig. 3C). The pH after treatment showed rela-
tively strong correlations with pressure and melanoidins generation (Table 3). However, we could not determine causality among them because of the difference in pH values of only about 0.2.

In summary, the heat treatment of an asparagine–glucose solution at pH 9.0 with <90 MPa pressure could efficiently generate melanoidins, with inhibition of acrylamide generation. This effect becomes pronounced as the Maillard reaction progresses.

In the present study condition (70°C, 60 and 90 MPa, up to 72 hours) using the asparagine–glucose mixture in TAPS buffer solution (pH 9.0), pressure treatments at 90 MPa inhibited acrylamide generation over time (Fig. 3A). However, melanoidins generation significantly increased with increasing pressure (Fig. 3B). We previously reported that pressure inhibited acrylamide generation but had no effect on melanoidins generation in a study of heat–pressure treatment (120°C, 100–300 MPa, 1 hour) using the same sample solution [16]. Notably, the amount of acrylamide generated in the control of the previous study’s condition was approximately 42-fold higher than that of the present study’s condition. Therefore, we infer that the marked difference between the two conditions on the degree of Maillard reaction progression resulted in differences in the effects of pressure on acrylamide and melanoidins generation.

The Maillard reaction is classified into three stages: the initial stage starts with the reaction of amino acids with carbonyl compounds, including reducing sugars; the intermediate stage involves the degradation of the Amadori rearrangement product, a Maillard reaction intermediate compound; and the advanced stage involves melanoidins generation via Strecker degradation and polymerization [26,27]. The effect of pressure on the reaction rate in a liquid phase is represented as activation volume ($\Delta V^\ne$) by the following equation.

$$\left( \frac{\partial \ln k}{\partial P} \right)_T = -\frac{\Delta V^\ne}{RT}$$

In the above equation, $k$ is the reaction rate constant, $P$ is pressure, $R$ is the gas constant, and $T$ is the absolute temperature. In calculating the constant $k$, the reactions of acrylamide and melanoidins formation could be well approximated by assuming a zero-order reaction, which has been frequently used to evaluate the Maillard reaction [28]. The negative $\Delta V^\ne$ reactions (i.e., those involving a decrease in the number of molecules and ionization) are promoted by pressure; in contrast, the positive $\Delta V^\ne$ reactions (i.e., those involving an increase in the number of molecules, such as bond cleavage) are inhibited [21]. The individual chemical reactions occurring in each stage of the Maillard reaction are affected by pressure according to their $\Delta V^\ne$, receiving different promotion or inhibition effects [29]. The values of $\Delta V^\ne$ calculated from Fig. 4 were $-21.6$ mL/mol for melanoidins and $+5.4$ mL/mol for acrylamide. This indicates that pressure acts in the direction that melanoidins formation is promoted and acrylamide formation is delayed. In the formation pathway of melanoidins, its intermediates Schiff base undergoes sugar dehydration and fragmentation following Amadori rearrangement [26]. Amadori rearrangement and decomposition of its rearrangement products are likely to be delayed by pressure [29]; on the other hand, Amadori rearrangement may be promoted in pH 10.2 condition [25]. In addition, subsequent reactions in which melanoidins are formed from decomposition products of Amadori rearrangement products are likely to be promoted by pressure, involving decreases in the number of molecules [29]. As a result of these associative reactions, the value of $\Delta V^\ne$ for melanoidins generation is expected to be negative. In contrast, acrylamide formation is expected to have the positive value of $\Delta V^\ne$ because acrylamide eventually generates through reactions involving an increase in the number of molecules from the Schiff base.

Also, the promoting effect of pressure on melanoidins generation can be observed only when the increase in browning is proportional to the progression of the Maillard reaction. In the present study, in which the Maillard reaction progressed moderately, this promoting effect of pressure could be observed because it appears that the degree of progression of the Maillard reaction agreed well with the increase in melanoidins. On the other hand, in the previous study [16], in which the Maillard reaction progressed rapidly, highly polymerized melanoidins might be formed and become partially insoluble; thus, the effect of pressure on melanoidins generation may have been underestimated. That is, the rapid progression of the Maillard reaction might cause the insolubilization of brown compounds. This insolubilization disrupts the proportional relationship between an increase in melanoidins and the progression of the Maillard reaction, leading to the inability to observe the effect of pressure on melanoidins generation. Unlike melanoidins generation, the inhibitory effect of pressure on acrylamide generation became pronounced with Maillard reaction progression. The reasons for this phenomenon are because acrylamide is originally a minor product and the effect of pressure on acrylamide genera-
tion is smaller than that on melanoidins generation.

The heat-pressure experiment of the asparagine and glucose solution containing cysteine at pH 9.0 showed that cysteine acted on the Maillard reaction involving acrylamide generation, and its action was partially influenced by pressure (Fig. 5A and B). Cysteine has an inhibitory effect on acrylamide formation, which is attributed to the competition reaction with asparagine and adduct formation with acrylamide [30,31]. This effect of cysteine was prominent in this study (Fig. 5A), and the inhibitory effect of pressure on acrylamide formation was observed at 90 MPa for 48 hours or more (Fig. 3A); therefore, the effect of pressure on the acrylamide formation reaction involving cysteine would not manifest under this reaction condition. On the other hand, cysteine influenced the effect of pressure on melanoidins generation (Fig. 5B). Cysteine is known to inhibit Maillard browning, which is attributed to adduct formation with carbonyl compounds via its thiol group [32]. However, cysteine generates low molecular weight pigments via the Maillard reaction [32]. It can be deduced that the Maillard reaction–related products involving cysteine cause other chemical equilibria and lead to a change in the influence of pressure on melanoidins formation. Unfortunately, we were unable to determine the mechanism by which the slight increase of pH in treated samples containing cysteine affected the degree of progression of the Maillard reaction.

We reported that cysteine inhibits acrylamide generation in a NCS solution, a model food that tends to produce acrylamide [22]. We then examined the inhibition of acrylamide generation in the NCS solution by applying pressure and adding cysteine. The cysteine–free NCS solution and the NCS solution with a low cysteine concentration treated at 70°C for 24 hours showed increased acrylamide levels compared with the control (Fig. 6A). We previously reported that under the heat-pressure condition (≤300 MPa, 120°C, 1 hour), an asparagine–glucose mixture in an MES buffer at pH 5.0 generated much less acrylamide and melanoidins than in a TAPS buffer at pH 9.0 [16]. This finding indicates that the condensation reaction between asparagine and glucose is unlikely to arise in mildly acidic conditions; therefore, acrylamide resulting from this reaction would be negligible in the NCS solution at a pH of approximately 5.5. Namely, NCS contains the initial reactants, asparagine and reducing sugars, and would contain acrylamide–related compounds, i.e., intermediates, adducts, and asparagine–reactive carbonyl compounds [33–40]. These compounds probably formed acrylamide in the NCS solutions.

On the other hand, cysteine exerted a remarkable decreasing effect on acrylamide in the NCS solutions under pressures of 0.1 and 90 MPa (Fig. 6A). This effect can be explained by the competition with asparagine and the adduct formation with acrylamide mentioned above [30,31]. Also, it is considered that cysteine can react with the above-mentioned acrylamide–related compounds to inhibit acrylamide formation. In contrast, the impact of pressure on acrylamide generation in the NCS solutions was much weaker than that of cysteine. Also, pressure promoted acrylamide generation in cysteine–free NCS solutions, but retarded the generation in the NCS solution with a high cysteine concentration. These results suggest that in NCS solutions, pressure promotes acrylamide formation from acrylamide intermediates while at the same time decreasing acrylamide by enhancing the effect of cysteine. As previously mentioned, pressure acts on individual reaction according to the $\Delta V^\pm$. When an NCS solution contains a high concentration of cysteine, the pressure would totally act on the inhibitory effect on acrylamide formation rather than on the promotion effect.

The treatment with pressure and cysteine tended to inhibit browning in NCS solutions, however, the degree of browning varied little between the untreated and the treated NCS solutions (Fig. 6B). This result indicates that the Maillard reaction leading to melanoidins formation hardly progressed in the NCS solutions treated at 70°C for 24 hours, which contrasts with acrylamide formation. On the other hand, during the preservation of NCS from production to experiment under ambient temperature, the Maillard reaction progresses, and the degree of coloring rises, but acrylamide content declines [41,42]. Regarding the relationship between browning degree and acrylamide generation, in general, the color tone of carbohydrate–rich processed foods can be used as a criterion of acrylamide level, while the behavior of acrylamide in NCS indicates that the brown color of NCS cannot be used as a criterion of acrylamide level. Moreover, the antioxidative activity of cysteine [42] may reduce the brown pigment of NCS, leading to inhibition of browning. This pigment contains mainly melanoidins and caramel colorants, as well as partly oxidatively polymerized phenolic compounds and low–molecular–weight colorants [43,44]. Furthermore, the inhibitory effect of pressure on browning under acidic conditions may be involved in a decrease in the degree of browning [17,18].
The treatment with pressure and cysteine lowered the pH of the NCS solutions (Fig. 6C). This pH drop is presumed to be due to oxidation of the thiol group in cysteine.

In summary, acrylamide generation by moderate heating in NCS solutions can be inhibited by cysteine. The application of pressure combined with cysteine addition might further inhibit acrylamide generation and browning of NCS.

5. Conclusions

The moderate heat treatment of an equimolar asparagine–glucose mixture in an aqueous alkaline solution under pressure (<100 MPa) showed promotion of the Maillard reaction and relative inhibition of acrylamide generation. Cysteine strongly inhibited acrylamide generation in the sample as mentioned above and in the NCS solutions. Especially, cysteine lowered the amount of acrylamide originally contained in NCS. The effect of pressure on NCS solutions was smaller than that of cysteine; however, it was found that their combination could inhibit acrylamide generation and browning. These findings would help to elucidate acrylamide generation and the Maillard reaction during fermentation and aging under medium high hydrostatic pressure.

References

1) International Agency for Research on Cancer; Acrylamide. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 60, 289-483 (1994).

2) E. Tareke, P. Rydberg, P. Karlsson, S. Eriksson, M. Törnqvist; Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J. Agric. Food Chem., 50, 4998-5006 (2002).

3) T. Gomyo, M. Miura; “Melanoidin in Foods: Chemical and physiological aspects” (in Japanese). Journal of Japanese Society of Nutrition and Food Science, 36, 331–340 (1983).

4) J. A. Rufián–Henares, F J. Morales; Functional properties of melanoidins: In vitro antioxidant, antimicrobial and antihypertensive activities. Food Res. Int., 40, 995–1002 (2007).

5) FAO and WHO; “Safety evaluation of certain contaminants in food.” WHO Food Additives Series 55, FAO Food and nutrition paper 82, the 64th meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2006, p. 1.

6) FAO and WHO; “Safety evaluation of certain contaminants in food.” WHO Food Additives Series 63, FAO JECFA Monographs 8, the 72nd meeting of the Joint FAO/WHO Expert Committee on Food Additives, 2011, p. 1.

7) A. Sasagawa, M. Kinefuchi, A. Yamazaki, A. Yamada; “High pressure bioscience” (in Japanese), R. Hayashi, S. Kunugi, S. Shimada, A. Suzuki ed., San-ei Shuppan, Kyoto, Japan, 1994, p. 336.

8) A. Yamazaki, M. Kinefuchi, K. Yamamoto, A. Yamada; “Physical properties and fine structure of grains of high-pressure–treated rice after cooking” (in Japanese). Rev. High Pressure Sci. Technol., 5, 168–178 (1996).

9) M. Kinefuchi, M Sekiya, A. Yamazaki, K. Yamamoto; “Accumulation of GABA in brown rice by high pressure treatment” (in Japanese). J. Jpn. Soc. Food Sci. Technol., 46, 323–328 (1999).

10) M. Kinefuchi, M. Sekiya, A. Yamazaki, K. Yamamoto; “Change in viable bacteria count in brown rice containing accumulated GABA by high pressure treatment, and properties of processed brown rice” (in Japanese). J. Jpn. Soc. Food Sci. Technol., 46, 329–333 (1999).

11) A. Sasagawa, Y. Naiki, S. Nagashima, M. Yamakura, A. Yamazaki, A. Yamada; “Process for producing brown rice with increased accumulation of GABA using high-pressure treatment and properties of GABA-increased brown rice” (in Japanese). J. Appl. Glycosci., 53, 27–33 (2006).

12) A. Kobayashi, M. Kawamura, E. Ohara, M. Ogino, J. Hoshino; “Application of high-pressure treatment to sterilization of foods” (in Japanese). Rev. High Pressure Sci. Technol., 24, 48–51 (2014).

13) M. Ogino, T. Nishiumi; “Sterilization of heat-resistant spores by a combination of high-pressure and subsequent heat treatment” (in Japanese). Rev. High Pressure Sci. Technol., 25, 334–342 (2015).

14) M. Ogino, T. Nishiumi; Control of the generation time of microorganisms by long-term application of hydrostatic pressure of 50 MPa or less. Food Sci. Technol. Res., 24, 289–298 (2018).

15) T. Okazaki, K. Noguchi; “The way of autolytic hydrolysis under hydrostatic pressure and development of its equipment” (in Japanese). Jpn. J. Food Eng., 9, 239–250 (2008).

16) A. Kobayashi, S. Gomikawa, A. Oguro, S. Maeda, A. Yamazaki, S. Sato, H. Maekawa; The effect of high hydrostatic pressure on acrylamide generation in aqueous reaction systems using asparagine and glucose. J. Food Sci. Technol. Res., 25, 587–596 (2019).

17) J. M. Ames; Application of the Maillard reaction in the food industry. Food Chem., 62, 431–439 (1998).

18) V. M. Hill, D.A. Ledward, J. M. Ames; Influence of high hydrostatic pressure and pH on the rate of Maillard browning in a glucose-lysine system. J. Agric. Food Chem., 44, 594–598 (1996).
19) T. Tamaoka, N. Itoh, R. Hayashi; High pressure effect on Maillard reaction. Agric. Bio. Chem., 55, 2071–2074 (1991).
20) K. De Vleeschouwer, I. Van Der Plancken, A. Van Loey, M. E. Hendrickx; The effect of high pressure-high temperature processing conditions on acrylamide formation and other Maillard reaction compounds. J. Agric. Food Chem., 58, 11740–11748 (2010).
21) M. Nakahara; “High pressure science for food” (in Japanese), R. Hayashi ed., San-ei Shuppan, Kyoto, Japan, 1991, p. 41.
22) A. Kobayashi, S. Gomikawa, A. Oguro, S. Maeda, A. Yamazaki, S. Sato, H. Maekawa; Effects on acrylamide generation under heating conditions by addition of lysine and cysteine to non-centrifugal cane sugar. J. Food Sci. Technol. Res., 26, 673–680 (2020).
23) J. J. Knol, W. A. M. Van Loon, J. P. H. Linssen, A. L. Ruck, M. A. J. S. Van Boekel, A. G. J. Voragen; Toward a kinetic model for acrylamide formation in a glucose-asparagine reaction system. J. Agric. Food Chem., 53, 6133–6139 (2005).
24) G. Maeda, I. Shimoji, T. Tedokon, H. Shimoji, K. Uechi, H. Ishimine, M. Sunagawa, J. Chinen, K. Degi, K. Miyagi, T. Ogi; “Comparison between non-centrifugal brown sugar (Kokuto) manufactured using cane juice and that manufactured using cane juice with cane top juice” (in Japanese). Okinawa Prefectural Agricultural Research Center Kenkyu Houkoku, 12, 14–20 (2018).
25) F. J. Moreno, E. Molina, A. Olano, R. Lopez-Fandino; High pressure effects on Maillard reaction between glucose and lysine. J. Agric. Food Chem., 51, 394–400 (2003).
26) H. Nursten; “The Maillard Reaction: Chemistry, Biochemistry and Implications.” The Royal Society of Chemistry, Cambridge, UK, 2005, p. 2.
27) S. I. F. S. Martins, W. M. F. Jongen, M. A. J. S. Van Boekel; A review of Maillard reaction in food and implications to kinetic modelling. Trends Food. Sci. Technol., 11, 364–373 (2001).
28) K. Kawai, T. Hagiwara, R. Takai, T. Suzuki; “Effect of reducing sugar on the Maillard reaction rate of freeze-dried food in the glassy state” (in Japanese). Japan J. Food Eng., 6, 59–64 (2005).
29) S. I. Martinez-Monteagudo, M. D. A. Saldaña; Chemical reaction in food systems at high hydrostatic pressure. Food Eng. Rev., 6, 150–157 (2014).
30) W. L. Claeyts, K. De Vleeschouwer, M. E. Hendrickx; Effect of amino acid on acrylamide formatoin and elimination kinetics. Biotechnol. Prog., 21, 1525–1530 (2005).
31) C. T. Kim, E.-S. Hwang, H. J. Lee; Reducing acrylamide in fried snack products by adding amino acids. J. Food Sci., 70, C354–C358 (2005).
32) M. Murata; “Food chemistry study on enzymatic browning and the Maillard reaction” (in Japanese). J. Jpn. Soc. Food Sci. Technol., 67, 1–12 (2020).
33) D. V. Zyzak, R. A. Sanders, M. Stojanovic, D. H. Tallmadge, B. Eberhart, D. K. Ewald, D. C. Gruber, T. R. Morsch, M. A. Strothers, G. P. Rizzi, M. D. Villagran; Acrylamide formation mechanism in heated foods. J. Agric. Food Chem., 51, 4782–4787 (2003).
34) M. Granvogl, J. Magnus, P. Koehler, P. Schieberle; Quantitation of 3-amino-propionamide in potatoes. A minor but potent precursor in acrylamide formation. J. Agric. Food Chem., 52, 4751–4757 (2004).
35) R. H. Stadler, F. Robert, S. Riediker, N. Varga, T. Davidek, S. Devaud, T. Goldmann, J. Hau, I. Blank; In-depth mechanistic study on the formation of acrylamide and other vinylogous compounds by the Maillard reaction. J. Agric. Food Chem., 52, 5550–5558 (2004).
36) M. Granvogl, P. Schieberle; Thermally generated 3-amino-propionamide as a transient intermediate in the formation of acrylamide. J. Agric. Food Chem., 54, 5933–5938 (2006).
37) I. Blank, F. Robert, T. Goldmann, P. Pollien, N. Varga, S. Devaud, F. Saucy, T. Huyhn–Ba, H. Stadler; “Chemistry and Safety of Acrylamide in Food. Advances in Experimental Medicine and Biology” vol. 561, M. Friedman, D. S. Mottram. Ed., Springer, New York, US, 2005, p. 171.
38) S. Ehling, M. Hengel, T. Shihamoto; “Chemistry and safety of acrylamide in food. Advances in Experimental Medicine and Biology” vol. 561, M. Friedman, D. S. Mottram ed., Springer, New York, US, 2005, p. 223.
39) K. Ishihara, A. Matsunaga, T. Miyoshi, K. Nakamura, T. Nakayama, S. Ito, H. Koga; Formation of acrylamide in a processed food model system, and examination of inhibitory conditions. J. Food Hyg. Soc. Japan, 46, 33–39 (2005).
40) K. Tsutsuimiuchi, M. Hibino, M. Kambe, N. Okajima, M. Okada, J. Miwa, H. Taniguchi; Effect of carbohydrates on formation of acrylamide in cooked food models. J. Appl. Glycosci., 52, 219–224 (2005).
41) N. Hirose, G. Maeda, K. Takara, K. Wada; “Changes in the physicochemical and flavor characteristics of the Okinawan brown sugar “kokuto” during storage at ambient temperature” (in Japanese). Food Preserv. Sci., 41, 253–259 (2015).
42) S. Honda, T. Masuda; “Polyphenols: Functional chemicals based on their chemical reactions, from antioxidation to inter-substance reactions” (in Japanese). Kagaku To Seibutsu, 53, 442–448 (2015).
43) K. Ujihara, M. Yoshimoto, K. Wada, M. Takahashi, I. Suda; “Enhancement of DPPH–radical scavenging activity in heat–processed sugarcane Molasses” (in Japanese). J. Jpn. Food Eng. 2021 Japan Society for Food Engineering
Soc. Food Sci. Technol., 60, 159–164 (2013).

44) W. R. Jaffé; Nutritional and functional components of non centrifugal cane sugar: A compilation of the data from analytical literature. J. Food Composition and Analysis, 43, 194-202 (2015).

**URLs cited**

i) www.maff.go.jp/j/syouan/seisaku/acrylamide/a_gl/pdf/131127_acrylamide_full.pdf (Jan. 23, 2014)

ii) www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_15-22.pdf (Oct. 31, 2012)

iii) www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_23-24_.pdf (Dec. 29, 2014)

iv) www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_25-26.pdf (Sep. 24, 2020)

v) www.maff.go.jp/j/syouan/seisaku/risk_analysis/survei/pdf/chem_27-28.pdf (Sep. 24, 2020)

vi) www.maff.go.jp/j/syouan/seisaku/papers_posters/pdf/106th_eisei1.pdf (Apr. 28, 2015)
静水圧とシステイン添加によるアクリルアミドの生成抑制

小林 篤1,2†, 五味川里子2, 小黒麻美2, 山﨑 彬2, 佐藤眞治3, 前川博史1

1長岡技術科学大学, 2越後製菓株式会社総合研究所, 3新潟薬科大学応用生命科学科

100 MPa未満の静水圧がアクリルアミドの生成とメイラード反応に及ぼす効果を、pH 9.0の等モル濃度のアスパラギン−グルコース水溶液を用いて検討した。常圧、60または90 MPaの圧力下で70℃、72時間までの反応を行い、アクリルアミドの生成量、メラノイジンの生成量、反応後のpHを測定した。メラノイジンの生成との比較によって、アクリルアミドの生成は、圧力保持により相対的に抑制されることが明らかとなった。また、同試料にシステインを添加して90 MPaの圧力下で70℃、24時間反応させた場合、圧力保持の有無に関らず、アクリルアミド生成が著しく抑制された。上述の結果に基づいて、圧力保持とシステイン添加によるアクリルアミド生成抑制効果を、アクリルアミドを比較的多く含む黒糖（Non-centrifugal cane sugar, NCS）水溶液（pH 5.5）を用いて同様の加圧加熱反応により検証した。システイン添加は、NCS水溶液の含有するアクリルアミドを低減し、加熱反応に伴うアクリルアミド生成を抑制した。また、NCS水溶液の圧力保持はアクリルアミドの生成を促進したが、高濃度のシステイン共存下での圧力保持は、アクリルアミドの低減を促進した。これらの結果から、食品の加工で起こるアクリルアミド生成やメイラード反応は、静水圧やシステインの添加で制御できる可能性が示唆された。