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Effect of Combination of Heating and Pressurization on Browning Reaction of Glucose-Glycine Solution and White Sauce

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The effect of the combination of heating and pressurization on Maillard reaction was investigated using a model solution of glucose and glycine, and a white sauce. The apparent browning rates were calculated from the relationship between the treatment time and the absorbance of the model solution at 430 nm. The apparent activation volume was estimated from the relationship between the apparent browning rate and the pressure, and the apparent activation energy estimated from the rate and the temperature. High hydrostatic pressure (HHP) up to 400 MPa retarded the Maillard reaction in a temperature range of 100–115°C. The apparent activation volumes fell into 3.8–4.5 cm^3·mol^{-1} in a temperature range of 100–115°C, and the apparent activation energies into 94.3–105.3 kJ·mol^{-1} in a pressure range of 0.1–400 MPa. The HHP treatment also suppressed the browning of the white sauce, which was heated at 115°C for 30 min under 300 MPa.

Keywords: pressure, high hydrostatic pressure, browning, Maillard reaction, white sauce

Browning of food components (sugars and amino acids) due to Maillard reaction brings favorable colors and smell to foods, but this reaction also deteriorates food quality in many cases of food production. It is known that processing conditions like temperature, pH as well as constituents of the ingredients affect the Maillard reaction. The reaction progresses in two stages, one of which is the condensation between carbonyl and amino compounds, and the other the browning reaction (Namiki, 1985). Tamaoka *et al.* (1991) showed that the Maillard reaction was suppressed by high hydrostatic pressure (HHP), which inhibited the browning reaction considerably more than the condensation reaction. HHP is, therefore, considered to be one of the important factors controlling the Maillard reaction. However, the effect of HHP treatment on the Maillard reaction due to heating has not been determined in the temperature range practically used by the food industry. In this context, we have studied the usefulness of thermal treatments combined with HHP in a temperature range higher than 100°C as a new technique for food processing from the viewpoint of food quality and sterilization (Okazaki *et al.*, 1994, 2000).

The objective in this study was to clarify the effects of HHP treatment on the Maillard reaction in a temperature range of 100–115°C. The reaction was tested using a model solution of glucose and glycine, and applicability of the results was confirmed using a white sauce as one of the model foods.

Materials and Methods

Preparation of model samples The model solution used was composed of glucose, one of the reducing sugars, and glycine as the amino acid (Guaranteed Grade, Wako Pure Chemical, Ltd., Tokyo). Both 1 M glucose and 1 M glycine were dissolved in 1000 ml of distilled water, and the solution (pH 5.6) was used for experiments as it was. A white sauce was made from milk (1000 ml), flour (100 g) and butter (100 g). All ingredients were mixed gradually, then heated gently with stirring by hand. The white sauce was frozen at −30°C, then, immediately before use in the experiment, it was thawed and diluted with milk to double its volume; glucose (1 wt%) and glycine (1 wt%) were then added to determine the effect of pressure on the browning. Fifty grams of the model white sauce was packed in a pouch (polyethylene film, 50×100 mm) for tests.

Pressurizing apparatus The pressurizing apparatus previously described was used for the experiments of the model solution (Okazaki *et al.*, 1994). The pressure vessel, a part of the pressure apparatus, was 8 mmϕ×300 mm in size and was directly heated in an oil bath after the model solution in the vessel was pressurized. Any amount of model solution in the vessel could be treated up to 500 MPa and up to 120°C simultaneously. For the white sauce experiments, a pressurizing-heating apparatus (Hikarikouatu-kiki, Hiroshima), capable of simultaneously pressurizing up to 400 MPa and heating up to 115°C was used. The pressure chamber was 100 mmϕ×300 mm in size and the pressurized medium (water) in the chamber was heated by an inner heater (200 W) and an outer one (400 W).

Pressurizing procedure One and two-tenths milliliters of the model solution was poured into a sample container (a 4 mm ϕ×100 mm silicone rubber tube sealed at one end) without air bubbles, and the open end of the container was stopped with a silicone rubber plug. This container was inserted into the pressure vessel made of stainless steel. After the required pressure was applied to the model solution in the container, the pressure vessel was placed for 5 min in a water bath (25°C) to make sure that the initial temperature of each sample was regulated to the same value. After this procedure, the pressure vessel was placed in an oil bath (OH-50, TAITEC Ltd.) at 100–115°C±0.1°C for 5 to 35 min. In the initial period of heating, the pressure increased

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to 20–30 MPa due to the thermal expansion of the water in the vessel. Therefore, the pressure was adjusted by controlling the valve so as to keep the required pressure. After heating by HHP treatment, the pressure vessel was transferred to the water bath again for 5 min to cool the sample, and the sample container was removed from the vessel after atmospheric pressure was restored by opening the valve. The temperature and pressure history curves of the solution during the heating with HHP treatments were almost the same as those shown in the previous report (Okazaki *et al.,* 1994). It took about 30 s to reach 400 MPa and about 15 s to return to atmospheric pressure, and about 300 s was required to reach 100°C, as an example of the heating with HHP treatments.

Four pouches of the white sauce were placed in the pressure chamber. The pressurized medium was heated to 90°C beforehand so that the treatment temperature (115°C) could be reached as soon as possible. Then, the HHP treatment and heating were applied to the samples at the same time. It took about 1.5 min to reach 350 MPa, and 10 min to reach 115°C.

Measurement of the browning rate and the change in color The browning value of each model solution was evaluated from the absorbance at 430 nm. The change in color of the white sauce was measured from the surface of the transparent pouches three times using the color-difference meter (Minolta, CR-100), and the mean values of *L* (lightness; 0 to 100, black to white), *a* (chromaticity; +, red; −, green), *b* (chromaticity; +, yellow; −, blue), and ΔE (color difference between pre-heating and heating with HHP or without HHP) were obtained.

Results and Discussion

Figure 1 shows the effect of pressure on browning behavior of a glucose-glycine solution at various temperatures. The browning value increased considerably with increase of temperature at the same pressure. For example, at atmospheric pressure (0.1 MPa), the value of 0.28 at 100°C for 30 min increased to 6.8 at 115°C for 30 min. When the HHP treatment was added, however, the Maillard reaction was inhibited at each temperature depending on the pressure. This suppression of browning by pressure coincided with a previous study (Tamaoka *et al.*, 1991), though the heating temperature in their study was much lower than that used here, and the kinds of sugar and amino acid used were different. For 30 min at 100°C, the degree of browning at 400 MPa was about one tenth that at 0.1 MPa, whereas in the same treatment time at 115°C, the degree of browning at 400 MPa was one third that at 0.1 MPa, because the progress curves of browning at over 105°C do not obey the first-order equation as shown below.

The relationships between the treatment time and the logarithm of the browning values are also shown in Fig. 2. Only the curves at 100°C were straight (r=0.997–0.982) as shown in (a), indicating that the Maillard reaction at each pressure obeyed a first-order reaction equation at 100°C. The slope of the curves decreased with increase in pressure. However, other than 100°C, none of the browning curves was straight but became concave upward, thus the Maillard reaction did not obey the first-order kinetics. The browning rates therefore could be calculated only from the experimental results at 100°C by linear regression; they were 0.184 min^{-1} at 0.1 MPa, 0.154 min^{-1} at 100 MPa, 0.135 min^{-1} at 200 MPa, 0.117 min^{-1} at 300 MPa and 0.091 min^{-1} at 400 MPa. The logarithmic values of these browning rates were plotted linearly against pressure (r=0.995, not shown), and the activation volume estimated from the slope of the linear curve was 5.22 cm^3·mol^{-1}. The positive value of the activation volume indicates that the Maillard reactions at 100°C were suppressed by

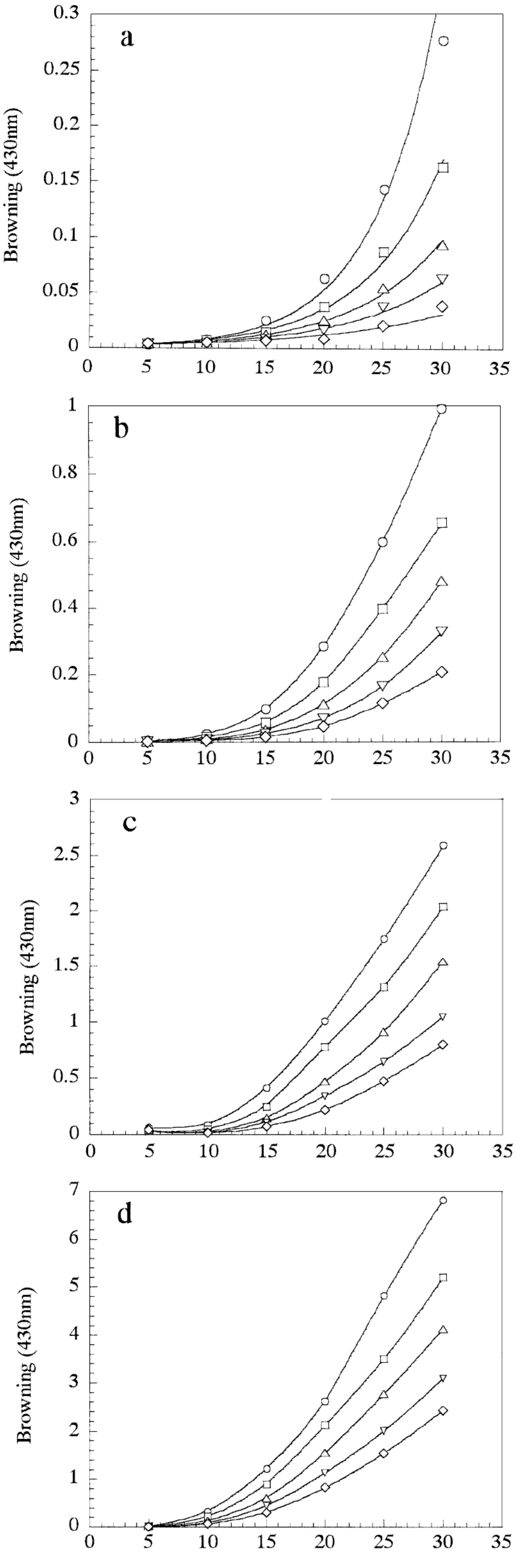

Fig. 1. Effect of pressure on browning behavior of glucose-glycine solution at various temperatures. ○, 0.1MPa; □, 100MPa; △, 200MPa; ▽, 300MPa; ◇, 400MPa. a, 100°C; b, 105°C; c, 110°C; d, 115°C.

applying pressure.

For the reaction temperatures higher than 105°C, the Maillard reaction did not obey the first-order equation as shown in Fig.2, and therefore, linear regression could not be applied to estimate the browning rate. The time required to reach the browning value of 0.1 at each temperature and pressure was then determined as in Fig.1, and abbreviated as $t_{0.1}$. We compared the effect of pressure on the Maillard reaction by using the reverse value of $t_{0.1}$ ($1/t_{0.1}$) instead of the real browning rate in this study.

The relationships between pressure and apparent browning rate are shown in Fig. 3. Curves of the relationship became linear in the temperature range from 100°C to 115°C, and were almost parallel. The apparent activation volumes calculated from the curves were within 3.8–4.5 $cm^3 \cdot mol^{-1}$ as shown in Table 1, though 4.5 $cm^3 \cdot mol^{-1}$ at 100°C was slightly less than the value (5.2 $cm^3 \cdot mol^{-1}$) that was calculated from the browning rate estimated from each linear curve in Fig.2 (a). Positive values of the

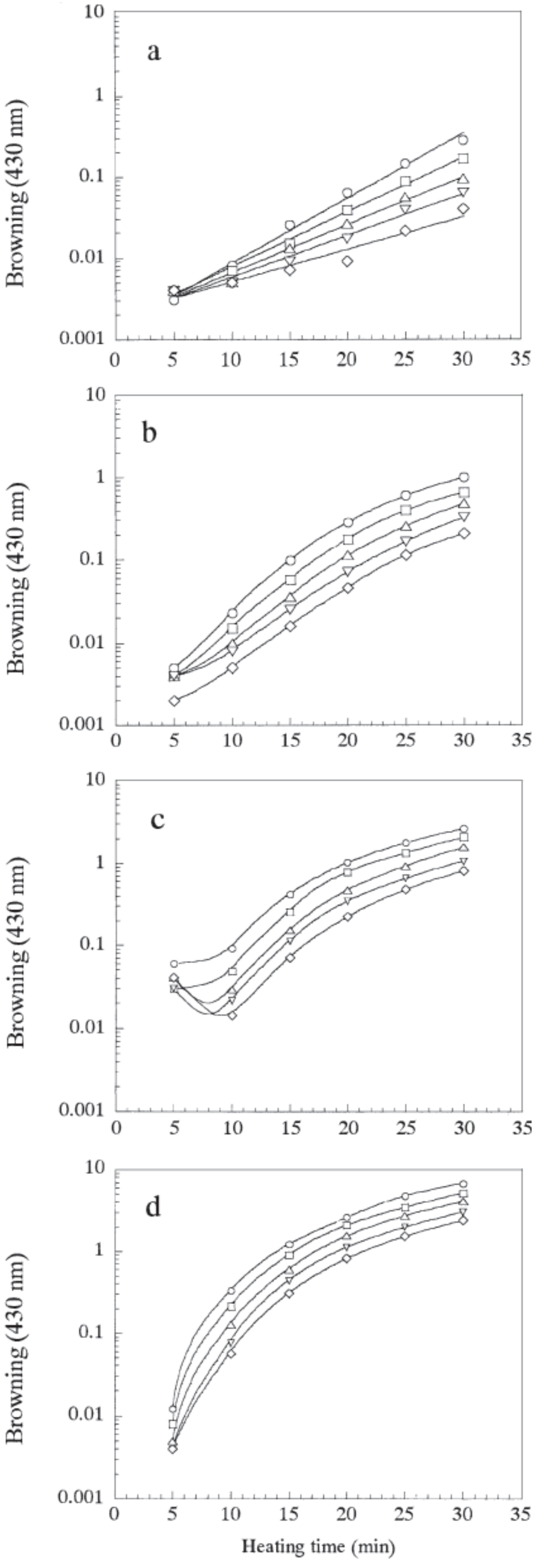

Fig. 2. Relationship between pressure and apparent browning value on glucose-glycine solution at various temperatures. Symbols are shown in Fig. 1.

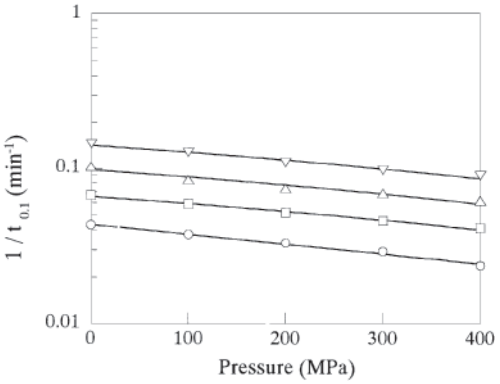

Fig. 3. Relationship between pressure and apparent browning rate in a temperature range of 100–115°C. ○, 100°C; □, 105°C; △, 110°C; ▽, 115°C.

Table 1. Apparent activation volumes of browning on the glucose-glycine solution (pH 5.6) in a pressure range of 0.1–400 MPa.

Temperature (°C)	Activation volume ($cm^3 \cdot mol^{-1}$)	A (min^{-1})	r
100	4.47[a] (5.22[b])	0.0436[a] (0.185[b])	0.995
105	3.77	0.0663	0.999
110	3.86	0.0979	0.983
115	3.94	0.1422	0.996

[a] The values were estimated from each linear curves in Fig. 3.

[b] The value was calculated from the browning rates estimated from each linear curve in Fig. 2 (a).

A, apparent browning rates at 0 MPa; r, correlation coefficient.

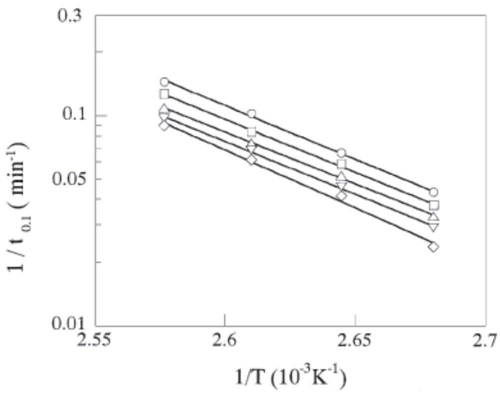

Fig. 4. Arrhenius plots of apparent browning rates in a pressure range of 0.1–400 MPa. ○, 0.1; □, 100; △, 200; ▽, 300; ◇, 400 (MPa).

Table 2. Apparent activation energies of Maillard reaction on the glucose-glycine solution (pH 5.6) in a temperature range of 100–115°C.

Pressure (MPa)	Activation energy (kJ/mol^{-1})	A ($\times 10^{12}$·min^{-1})	*r*
0.1	97.4[a]	1.865[a]	0.999
100	96.3	1.149	0.999
200	94.3	0.528	0.999
300	96.1	0.869	0.999
400	105.3	13.811	0.997

[a] The values were calculated from each linear curves in Fig. 4.
A, frequency factor; *r*, correlation coefficient.

apparent activation volumes showed that the Maillard reaction was restrained by pressure. The browning value evaluated from absorbance at 430 nm in the present study is believed to be a product of the total reaction through the two steps of the Maillard reaction, the condensation reaction and the browning reaction. Therefore, the pressure might affect either of the two reactions or both of them. Tamaoka *et al.* (1991) estimated the two activation volumes separately from the condensation reaction and the browning reaction; the former was 1.3–8.9 cm^3·mol^{-1} and the latter was 12.8–27.0 cm^3·mol^{-1}. The activation volumes (3.8–4.5 cm^3·mol^{-1}) obtained in the present study were within the former values (1.3–8.9 cm^3·mol^{-1}).

Figure 4 shows the Arrhenius plots of the apparent browning rates in a pressure range of 0.1–400 MPa. Each curve became linear and almost parallel in a temperature range of 100–115°C. Thus, the Maillard reaction of glucose-glycine solution obeyed the Arrhenius equation on heating with the HHP treatments. Table 2 shows apparent activation energies of the Maillard reaction calculated from the curves shown in Fig. 4. Values of the activation energies (94.3–105.3 kJ·mol^{-1}) were almost the same in the pressure range of 0.1–400 MPa. Therefore, the activation energies of Maillard reaction might be almost unchanged even though the pressurization increases to 400 MPa. The values of the activation energy of the Maillard reaction previously reported were 105–209 kJ·mol^{-1} (Thijssen & Kerkhof, 1977). The activation energy of the Maillard reaction obtained in the present study was within but among the lowest of those reported values.

In order to make sure that the suppression of the Maillard reaction by the HHP treatment can be applied to practical food processes, we tested the browning of a white sauce as a model food. The color difference and photographs of the sauce are shown in Table 3 and in Fig. 5, respectively. The white sauce (b) heated with the HHP treatment browned somewhat more than the pre-heated sauce (a), whereas that (c) heated without the HHP treatment browned considerably more than the sauce showing in (b). Thus, the appearance of the white sauce clearly showed a difference in color, depending on heating with or without HHP treatment. When the white sauce was heated without this HHP treatment, the *L*-value (lightness) decreased significantly, but decreased only slightly with the HHP treatment. The *a*-value for the "heating with HHP" retained its original value, but the value for "heating without HHP" increased considerably to a positive value from one that was originally negative. The increase in "*a*-value" toward the positive side indicates an increase in redness. Furthermore, the ΔE of the white sauce heated with HHP treatment was considerably less than that of white sauce heated without this treatment. In addition, in a sensory test the white sauce heated without HHP had a strong heated odor compared with that heated with HHP (data not shown). Therefore, the browning of the white sauce by heating was suppressed sig-

Table 3. Browning of white sauce by heating with HHP treatment and without HHP treatment.

Sample	*L*	*a*	*b*	ΔE*
Pre-heating	77.8	−2.8	6.0	—
Heating with HHP	75.0	−2.7	10.4	5.2
Heating without HHP	69.0	0.4	16.0	13.7

White sauce containing 1% glucose and 1% glycine was treated at 0.1 MPa or 115°C, 30 min. ΔE* is color difference between pre-heating and heating with or without HHP.

(a) (b) (c)

Fig. 5. Photographs of white sauce after heating with and without HHP treatment. (a), pre-heated; (b), after heating with HHP treatment; (c), after heating without HHP treatment.

nificantly by the HHP treatment. "Heating with HHP" treatment is able to inhibit thermal change in the original color of other foods as well as white sauce. From these experimental results, it is clear that heating with this treatment suppressed not only the Maillard reaction of glucose and glycine, but also browning of a white sauce. In addition, it is reported that the heating with HHP inactivates bacterial spores (Okazaki *et al*., 2000), and remains food qualities such as color, nutrient components (Shimada *et al*., 1990) and hardness of root vegetables (Okazaki *et al*., 1998). Therefore, this heating with HHP treatment will have capability as a new technique for food processing.

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