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Effect of High Hydrostatic Pressure Combined with pH and Temperature on Glucose/Fructose–Leucine/Lysine/Glutamate Browning Reactions

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Effect of high hydrostatic pressure (100 MPa) combined with pH (6.0, 7.0 and 8.0) and temperatures (80 °C and 90 °C) on Maillard reaction was investigated via the sugar (glucose or fructose)–amino acid (leucine, lysine or glutamate) solution models. Overall intermediate and final browning products produced were spectrophotometrically measured, and 5–(hydroxymethyl)–2–furfural (HMF) content was gas–chromatographically detected. Increases of pH and temperature accelerated the production of intermediate and final products of all solutions, while high pressure treatment suppressed the reaction. HMF was the most abundant compound among the detected compounds on chromatograms. Its content was also increased with the increase in temperature, but decreased in high pressure treatment. Effect of pH on the productions of intermediate and final compounds in glucose–amino acid systems was greater than in fructose–amino acid systems. Among three amino acids used, lysine was the most reactive amino acid in the productions of intermediate and final browning products, including production of HMF.

INTRODUCTION

Maillard reaction, that is a kind of non–enzymatic browning reaction, is basically generated from the reaction between reducing sugar and amino acid. This reaction plays an important role in the food–processes and in the qualities of final products. It is very easily induced by high temperature and weak basicity (Chen and Ho, 1999), then accompanied by the alterations of color and flavor. Not only formations of color pigments and volatile flavorants affect the sensory characteristic of foods but, nutritionally, unavailabilities of proteins and amino acids are also the negative outcomes from this reaction. Maillard reaction actually presents the advantages and is desired in some types of food products (e.g. soy sauce, coffee, bread or meat), but in most foods, it is estimated and placed on detrimental effects rather than beneficial effects (Ajandouz *et al.*, 2001).

Even though Maillard reaction has been known to be controlled by various conditions such as water activity, pH, temperature and types of sugar and amino acid, the reaction is still not completely understood because of complexity of reaction, diversities of reactants

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and products, and complication of reaction routes (Zamora *et al.*, 2000). Many researchers, to date, have attempted to study about Maillard reaction occurred from various types of sugar–amino acid under several conditions (Hill *et al.*, 1996; Tomaoka *et al.*, 1991), yet reactions generated from dissimilar reactants such as structural difference of sugar or acid–basicity of amino acid have not been performed and compared.

Until the present day, thermal treatment has been widely disseminated for microbial and enzymatic inactivation due to the ease of execution and high inactivation efficiency. This method, however, negatively influences the qualities of foods, for instance undesirable color and odor, losing shape and nutrition. For better preservations of original color, flavor and natural freshness, high hydrostatic pressurization has been interesting as the another alternative to heat treatment in diverse food–processes, such as blanching (inactivation of enzymes) or sterilization (destruction of microorganisms) (Schwarzenbolz *et al.*, 1996; Weemaes *et al.*, 1998). High pressure does not affect the covalent bond, thus flavor, color, nutrients and other fresh–like qualities remain in the food material more than using thermal treatment (Préstamo and Arroyo, 1998). Recently high pressure has been commercially exploited for the selected foods, and besides, combination with moderate temperature will enhance sterilization ability to be able to destroy the spores more effectively (Hill *et al.*, 1996).

Apart from inactivation of microorganisms, high pressure technique also influences the chemical and biochemical reactions, but a few researches have been carried out on the Maillard reaction (Hill *et al.*, 1999). The aim of this study, therefore, was to get the useful information on the effect of high hydrostatic pressure with several pH and temperatures on amino–carbonyl reactions occurred in structurally different sugar and (acidic–basic)–different amino acid model systems.

MATERIALS AND METHODS

Materials

L–Leucine, L(+)-glutamate monohydrate and L-(+)-lysine hydrochloride obtained from Wako Pure Chemical Industry, Ltd. (Osaka, Japan) were used as neutral-, acidic- and basic-amino acids, respectively. D-(+)Glucose and D-(-)fructose were from Nacalai Tesque, Inc. (Kyoto, Japan). Hydrochloric acid, sodium hydroxide, diethyl ether and sodium sulfate anhydrous were also obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Preparation of model solutions

Model solutions were prepared by dissolving glucose or fructose at 40% w/w with either leucine, glutamate or lysine at 2% w/w in distilled water. The pH of these solutions was adjusted to 6.0, 7.0 and 8.0 by addition of 0.2M hydrochloric acid and/or sodium hydroxide. Each solution was separated into two portions and both were put into plastic pouches. One part of samples was subjected to 80 and 90°C water bath for 20 hr as atmospheric treatment and another part of samples was subjected to 100 MPa high hydrostatic pressure treatment at the same temperatures and time interval.

High pressurization treatment

Temperature–controllable pressurization equipment (Yamamoto Suiatsu Kogyosho

Co., Ltd., Osaka, Japan) with a cylindrical pressure chamber (inside volume: 8 liters) was used. Treatment temperature was regulated by a digital temperature controller to $\pm 2^\circ\text{C}$ (type SR-62, Shimadzu Co. Ltd., Tokyo, Japan). The pressurization rate was 20 MPa/min, and decompression time from 100 MPa to 0.1 MPa was less than 20 sec.

ANALYTICAL PROCEDURES

Overall compound determination

Overall compounds were defined as intermediate stage products and final Maillard products. UV absorbable colorless compounds formed during intermediate stage and brown pigments formed during final stage were measured by UV-visible spectrophotometer (UV 1600) from Shimadzu at the absorbance 280 and 420 nm, respectively (Ajandouz *et al.*, 2001; Carabasa-Giribet and Ibarz-Ribas, 2000). Samples had been diluted for 400-fold and 100-fold before monitoring the intermediate and final products, respectively.

5-(Hydroxymethyl)-2-furfural (HMF) determination

HMF, the intermediate compound of Maillard reaction, was measured by gas chromatography (GC). Twenty five micro-liters of 0.1% 2-phenyl ethanol as an internal standard were put into the sample of 50 ml. HMF was extracted by liquid-liquid extraction with diethyl ether. The extracts were dried over anhydrous sodium sulfate for 3 hr and concentrated to about 50 μl . HMF was analyzed by GC and GC-mass spectrometer (GC-MS).

GC and GC-MS conditions

GC analysis was performed by Shimadzu GC-14A equipped with DB-5MS column ((5% phenyl) methylpolysiloxane coating, 30 m \times 0.25 mm (i.d.), 0.25 μm film thickness, J&W Scientific) and flame ionization detector. The column temperature was programmed from 60 to 230 $^\circ\text{C}$ at the rate of 4 $^\circ\text{C}/\text{min}$. Both injector and detector temperatures were 230 $^\circ\text{C}$. Flow rate of helium carrier gas was 0.8 ml/min. GC-MS analysis was performed via Hewlett Packard model 5890 seriesII GC coupled with electron impact mass spectrometer from JEOL AUTO MASS 50. Capillary column and GC-MS conditions were similar to GC analysis.

RESULTS AND DISCUSSION

Overall intermediate and final browning products

Relative productions of intermediate and final Maillard products from sugar-amino acid reactions under several conditions are shown in Fig. 1. All sugar-amino acid models at 90 $^\circ\text{C}$ produced more intermediate and final browning products than those at 80 $^\circ\text{C}$. The formation rates of colorless intermediates and brown pigments were increased with the increase in treatment temperature (Carabasa-Giribet and Ibarz-Ribas, 2000). As shown in Fig. 1, fructose without amino acid conspicuously produced the intermediate products at 90 $^\circ\text{C}$, moreover, it produced as much as fructose-leucine and fructose-glutamate whereas glucose free amino acid did not. Ajandouz *et al.* (2001) reported the

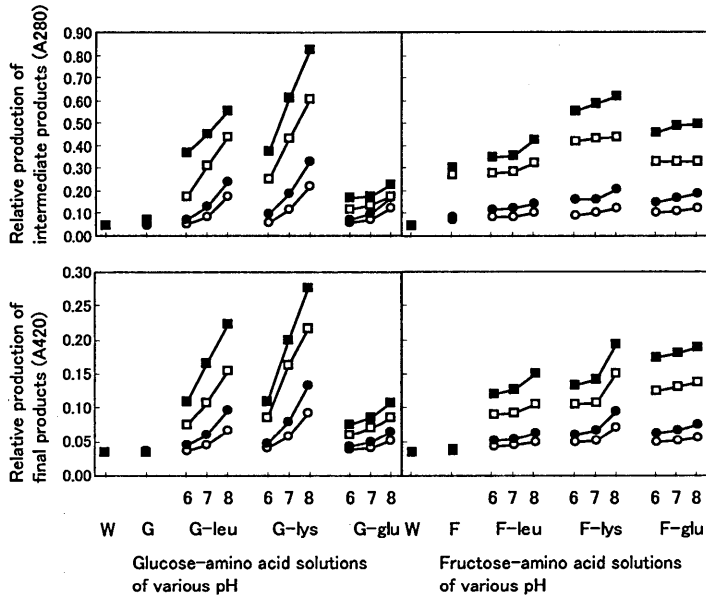


Fig. 1. Relative productions of intermediate and final browning products produced by several types of sugar–amino acid (W, water; G, glucose; F, fructose; leu, leucine; lys, lysine; glu, glutamate) at different pH (6.0, 7.0, 8.0) and temperatures (80 °C, circle symbol; 90 °C, square symbol) under either atmospheric pressure (close symbol) or 100 MPa pressure (open symbol).

progressive accumulation of the intermediate products from fructose heated alone initially occurred at pH 4.0, and the loss of fructose in this system was larger than in fructose–lysine system. Nevertheless, amount of intermediate products from fructose–lysine system was higher than that from fructose alone. In fact, monosaccharide such as pentose or hexose is possibly changed to furfural and/or furfural derivative after water molecules have been removed from their molecules by the action of heat and acid (Shibamoto, 1983). Monti *et al.* (2000) demonstrated that main contributor of color of heated lactose–*N*^ε–acetyllysine was the high molecular weight fraction, while in lactose free lysine low molecular weight fraction was the main responsible for color.

Fig. 1 shows the increase of solution pH promoted the intermediate and final browning formations. The enhancing behavior of relative productions of intermediate and final products in each sugar–amino acid solution by increase of pH was relatively same. Lower basicity compounds from deamination, fragmentation of sugars to acids and condensation between free amino acids and carbonyl groups are the cause of the decrease of solution pH during the reaction (Carabasa–Giribet and Ibarz–Ribas, 2000; Spark, 1969). Increasing pH value of solution, accordingly, would accelerate the transformation of reactants to intermediate and final browning products. Spark (1969) reported aldiosylamine, that is the product of initial reaction of sugar and amino acid, was more rapidly occurred in higher pH. Ajandouz *et al.* (2001) also stated an increase in pH of heated solutions

resulted in the increase in initial rate of degradation of fructose and lysine.

Effect of high hydrostatic pressure on the intermediate and final browning products is shown in Fig. 1. In every pH and temperatures, use of 100 MPa pressure resulted in suppression of intermediate and final browning productions. Decrease in the formation of Maillard volatiles by application of high pressure was previously demonstrated (Schwarzenbolz *et al.*, 2000). Tomaoka *et al.* (1991) reported amino–carbonyl reaction of α -alanine with glyceraldehyde was suppressed with the increase in pressure. The formation of nonvolatile products under high pressure, including Melanoidins, might proceed via the other pathways that produced fewer intermediate volatile compounds than common reaction (Hill *et al.*, 1999). Furthermore, retardation of degradation rate of the Amadori rearrangement products or advanced stage of the Maillard reaction under high pressure is also the convincing reason described up to now (Schwarzenbolz *et al.*, 2000; Hill *et al.*, 1999). In the biological system, there were some literatures reported that pH value of buffered solution was decreased by high pressure on account of the enhancement of weak acid dissociation (Molina–Futierrez *et al.*, 2002). This might also be responsible for the retardation of reaction. On the other hand, Hill *et al.* (1996) demonstrated pressure enhanced the browning rate at initial pH 8.0 and 10.1, but the effect was reversed at pH 6.5 and 5.1.

In general, Maillard reaction is well known that it is influenced by the participating reactants. Differences of absorbance between glucose and fructose with neutral-, acidic-, and basic-amino acids were observed in this work. Effect of pH on the intermediate and final products in fructose–amino acid solutions was small comparing with glucose–amino acid (Fig. 1), that is, intermediate and final browning products in glucose–amino acid systems were more drastically increased with high pH than in fructose–amino acid systems. This result might be attributed to the different reaction pathways between glucose and fructose. Glucose proceeds the Maillard reaction through Amadori rearrangement, whereas fructose goes through Heyns rearrangement. Different pathways generate different amounts as well as types of product. Among glucose–amino acid systems, glutamate showed the lowest absorbance in intermediate and final browning products at every conditions. Carabasa–Giribet and Ibarz–Ribas (2000) who dealt with the kinetics of color development in the systems of glucose with three types of amino acid systems postulated that glutamic acid was the low reactive amino acid for intermediate and brown pigment formations. The reactivity of used amino acids, in glucose systems, was ordered as follow: lysine > leucine > glutamate. Fructose–amino acid systems did not show the reactivity of amino acid as clear as glucose systems. Leucine seemed to be the least reactive amino acid, while lysine was still the most reactive. During Maillard reaction, H^+ generated from sugar fragmentation and other steps in sugar–lysine systems was more largely buffered than in –leucine and –glutamate systems because lysine, basic amino acid composing of 2 amino groups and 1 carbonyl group, has higher potential to stabilize the pH of solution. Intermediate and final products in lysine solution, therefore, would be produced in a larger amount than in other systems. However glutamate, in fructose solution, produced final browning products in a larger amount than lysine at pH 6 and 7 in 90 °C–treatments.

5–(Hydroxymethyl)–2–furfural (HMF)

HMF is the important colorless substance formed in the initial to intermediate stage

of Maillard reaction. It is classified into the intermediate product that can continuously enter the final stage and produces browning pigments. According to gas-chromatographic determination, HMF was a peak occupying the largest area on chromatograms. HMF formations from the glucose-amino acid and fructose-amino acid systems are shown in Fig. 2. Amount of produced HMF from glucose-leucine was as much as that from fructose-leucine. But with other amino acids, glucose systems produced the HMF in a smaller amount than fructose systems, particularly in pH 6 and 7 of lysine solutions. These coincided with the fact, that fructose is able to generate HMF more readily than glucose in acidic conditions (Spark, 1969). Among glucose-amino acid solutions, lysine was the most reactive in HMF production, and glutamate was the least. These causes might concern with the kinetic rate constant of HMF formation of glutamate and basic amino acid of lysine as prior mention.

High temperature induced, and high pressure retarded the production of HMF same as overall intermediate products. Carabasa-Giribet and Ibarz-Ribas (2000) showed the amount of HMF increased with raising temperature by following zero-order reaction. At pH 8 of sugar-lysine solutions, the amount of HMF became smaller than at pH 7, moreover smaller than at pH 6 in the fructose solution. These might be due to the transformation of intermediate substances in further reactions under severe conditions such as high temperature and high pH (Ajandouz *et al.*, 2001; Ames *et al.*, 2001). Fructose free amino acid also produced HMF, as intermediate compounds produced from fructose alone, and produced in a larger amount than fructose-leucine and fructose-glutamate. Reversible interaction which takes place between ketose sugar and amino acid is used as the reasonable quotation. Ajandouz *et al.* (2001) reported that fructose degradation was lowered in presence of lysine comparing with fructose alone.

A number of heterocyclic compounds in addition to HMF were also found in the sugar-amino acid solutions; for instance, imidazoles, furans, pyrroles, carboxylic acids, aldehydes (data was not shown). These great varieties of compounds were generated during initial degradation of sugar, and polymerization of intermediate compounds with

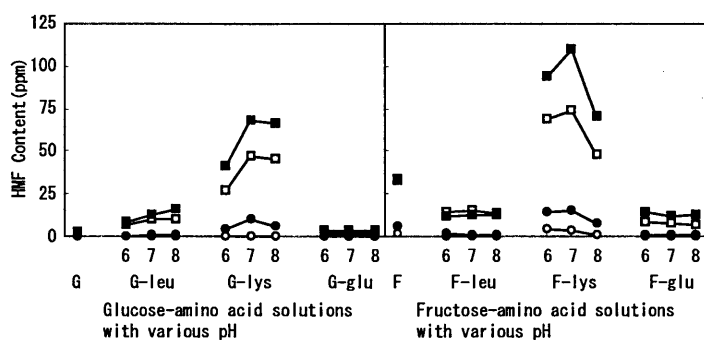


Fig. 2. 5-(Hydroxymethyl)-2-furfural produced by several types of sugar-amino acid (G, glucose; F, fructose; leu, leucine; lys, lysine; glu, glutamate) at different pH (6.0, 7.0, 8.0) and temperatures (80 °C, circle symbol; 90 °C, square symbol) under either atmospheric pressure (close symbol) or 100 MPa pressure (open symbol).

amino acid (Carabasa-Giribet and Ibarz-Ribas, 2000). Pyrroles are the origin of the color and important aroma compounds in the food (Zamora *et al.*, 2000; Chen and Ho, 1999). 2-Acetylpyrrole was an undetectable compound on any fructose-amino acid chromatograms. Furfural was a major component increasingly formed by low pH and high temperature (Ames *et al.*, 2001). 5-Methyl-2-furfural, another great furfural derivative, was not found in sugar-lysine solutions.

CONCLUSION

Maillard reactions occurred in sugar and neutral-, acidic-, basic-amino acid model systems were suppressed under high hydrostatic pressure, but accelerated by high pH value and high temperature. HMF, the greatest detectable compound, was also enhanced with high temperature, but inversely, lowered with high pressure. Glucose-amino acids were more sensitive to the change of pH than fructose-amino acids. Lysine used as the basic amino acid representative showed the greatest reactivity for browning reaction in both glucose and fructose systems.

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