The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control

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Summary. The color intensities was determined of Maillard reaction products (MRPs) prepared by heating each of five sugars (maltose, fructose, glucose, arabinose, and xylose) with each of 12 amino acids (aspartic acid, glutamic acid, alanine, leucine, isoleucine, valine, proline, serine, cysteine, phenylalanine, arginine, and lysine). The remaining percentages of glucose and rate of change of color intensity due to the addition of a metal ion and NaCl were monitored for nine MRPs that had been formed between glucose and each of nine amino acids (aspartic acid, glutamic acid, alanine, valine, serine, cysteine, phenylalanine, arginine, and lysine). Model MRPs were prepared in a block heater at 100°C for 1–12 h with the pH value controlled at 6.5. The resulting color intensity of each MRPs formed from the basic amino acids was greater due to the higher reactivity than those from the acidic amino acids. The remaining percentage of glucose in each MRPs from the basic amino acids was lower than those from the acidic amino acids. The MRPs from the nonpolar amino acids showed an intermediate color intensity and remaining percentages of glucose between those formed from the basic and acidic amino acids. Browning tended to be accelerated in the presence of metal ions, especially Fe^{2+} and Cu^{2+}, although it was affected by the property of the amino acid and heating time as well as by the type of metal ion. On the other hand, browning was greatly inhibited by a high concentration of NaCl.

Keywords: Maillard Reaction Products (MRP) – Browning – Melanoidin

Introduction

The Maillard reaction occurring between an amino acid or protein and a reducing sugar is a complicated reaction that produces a large number of Maillard reaction products (MRPs) such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds as melanoidin (Wijewickreme et al., 1997). This reaction commonly occurs during the cooking, preservation, and processing of foods. It is well known that the color of such fermented foods as soybean paste (Hondo, 1993) and soy sauce (Murata et al., 1995) is due to melanoidins. Soybean paste, which is widely used as a seasoning in Korea, Japan, and China, is prepared by inoculating *Aspergillus oryzae* and a mold into steamed soybean, adding 10% salt, and fermenting for a few months. Its palatable taste is due to free amino acids such as glutamic acid that are degraded from soybean protein during fermentation. The initial color of soybean paste is bright yellowish brown, and this changes to dark-brown with increasing preservation time. Such discoloration of soybean paste is the most influential factor in decreasing its desirability to consumer's (Yamabe, 1991; Hong et al., 1997). Hence, a method for inhibiting this browning reaction needs to be founded for use by soybean paste manufacture. According to the results of a study on the browning of soybean paste, the color change is dependent on *koji* (Hondo, 1993; Kwon et al., 1998) and accelerated by oxygen during preservation (Kwon et al., 1998). Temperature also has an influence on the browning rate (Hondo, 1993; Kwon et al., 1998).

It has been believed that the components of soybean paste responsible for browning should be investigated in order to study the inhibition of browning; however, these are difficult to find in a complex food system, so we need to examine the reaction using a simpler model system. Since the pH of such a model system substantially decreases during the reaction period (Homma et al., 1982; Wijewickreme et al., 1997; Morales and Jiménez-Pérez, 2001), it is necessary to match the pH value of the model system to that of the food being modeled. In practice, the decrease in pH value is less than 1 for soybean paste (Kim et al., 1999; Kim et al., 2000). The pH of the Maillard reaction is an important parameter in the

characteristics of the intermediates (Hayashi and Namiki, 1986; Ames and Apriyantono, 1993; Apriyantono and Ames, 1993; Bemis-Young et al., 1993; Ames and Apriyantono, 1994; Ames et al., 1997a; Ames et al., 1997b; Hidalgo et al., 1999), in addition to the reaction rate. Although the relationship between such factors as the type of sugar and amino compound (Ashoor and Zent, 1984; Hayashi and Namiki, 1986; Ajandouz and Puigserver, 1999) or metal ions (Gomyo and Horikoshi, 1976; Kato et al., 1981; Morita and Kashimura, 1991; Fallico and Ames, 1999) and browning have been studied, no data are available on the effect of the reactants on the browning that occurs under controlled pH condition.

In this study, we investigated how a model system incorporating a sugar, amino acid, metal ion, and NaCl would affect browning with the pH value controlled to 6.5, the same as that of soybean paste.

Materials and methods

Materials

L-aspartic acid, L-glutamic acid, L-alanine, L-leucine, L-isoleucine, L-valine, L-proline, L-serine, L-phenylalanine, L-arginine, D-maltose, D-fructose, D-arabinose, $FeCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $ZnCl_2$, $CaCl_2 \cdot 2H_2O$, and $AlCl_3 \cdot 6H_2O$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). L-cysteine, L-lysine, D-glucose, D-xylose, and NaCl were purchased from Wako Chemical Co. (Osaka, Japan). And HPLC-grade water (J. T. Baker, Phillipsburg, USA) was used.

Preparation of model MRPs

Equimolar (0.1 M) mixture of each of five sugars (maltose, fructose, glucose, arabinose, and xylose) and each of 12 amino acids (aspartic acid, glutamic acid, alanine, leucine, isoleucine, valine, proline, serine, cysteine, phenylalanine, arginine, and lysine) were dissolved in a 1 M citrate-phosphate buffer (pH 6.5). Test tubes (251×200 mm) containing 15 ml of each mixed solution were screw-sealed and heated in a block heater at $100°C$ for 1–12 h. After heating, the tubes were immediately cooled under tap water and stored in a refrigerator until needed, except for the MRPs with cysteine that were prepared immediately before use. In addition, a metal ion (0.2 mM) or NaCl (1 and 10%) was added to a mixed solution of glucose and each of nine amino acids (aspartic acid, glutamic acid, alanine, valine, serine, cysteine, phenylalanine, arginine, and lysine) at the final concentration, and each mixture heated for 3 h or 6 h under the same condition. Fe^{2+}, Cu^{2+}, Al^{3+}, Zn^{2+}, Mg^{2+}, and Ca^{2+} were used as metal ion.

Determination of color intensity

The color intensity of each of the 60 model MRPs prepared from the different combinations of the five sugars and 12 amino acids was determined with an HP 8435 spectrophotometer (Germany) as the absorbance at 420 nm against distilled water. The rate of change of color intensity caused by the addition of a metal ion or NaCl was also measured against the same MRP without the metal ion or NaCl as a control. The rate of change of color intensity (%) was expressed as [(absorbance of MRP with a metal ion or salt $\times$ 100/absorbance of control) − 100].

Determination of remaining percentage of glucose

The unreacted glucose in nine MRPs prepared by heating glucose with each of the nine amino acids for 3, 6, and 12 h was determined using HPLC (Jasco, Japan). A Jasco PU-987 pump connected to an RI detector (RI-930, Jasco, Japan) was used with a high performance carbohydrate column (4.6×250 mm, 4 μm particle size, Waters, USA). The mobile phase of acetonitrile:water (20:80, v/v) was delivered at a flow rate of 1.5 ml/min. Each MRP sample was diluted 10-fold, and 10 μl was injected. The remaining percentage (%) is expressed as (glucose content of MRP/glucose content of mixed solution before heating) $\times$ 100.

Statistical analysis

All experiments were carried out in duplicate, and Duncan's multiple range test in the SPSS statistical package (version 10.0) was used to detect significant differences ($p < 0.05$).

Results

Table 1 shows typical pH changes in the nine mixed solutions and MRPs from glucose with each of the nine amino acids (aspartic acid, glutamic acid, alanine, valine, serine, cysteine, phenylalanine, arginine, and lysine). Even before heating, the pH value of the mixed solution of glucose and arginine was the highest (6.75), while the values with aspartic acid and glutamic acid were the lowest (6.25). After heating for 12 h, the MRPs from arginine had the highest pH value of 6.52, whereas those of aspartic acid and glutamic acid had the lowest pH value of 6.10.

Figure 1 shows the color intensity of 60 MRPs heated for 3 h. The degree of contribution from the five sugars to browning was in the order of xylose > arabinose > glucose > maltose > fructose. Since lysine had the highest reactivity among the 12 amino acids, the color intensity of the MRPs from lysine was the highest while reacting with each kind of sugar (Fig. 1). The color intensity of the

Table 1. Change in pH value of nine mixed solutions and model MRPs from glucose with each of nine amino acids

Type of MRPs	Reaction time (h)		
	0	6	12
Aspartic acid	6.25	6.20	6.10
Glutamic acid	6.25	6.20	6.10
Alanine	6.50	6.39	6.34
Valine	6.50	6.30	6.23
Serine	6.50	6.31	6.27
Cysteine	6.50	6.38	6.35
Phenylalanine	6.50	6.30	6.25
Arginine	6.75	6.55	6.52
Lysine	6.50	6.30	6.25

Each value is the mean of two determinations

MRPs from the other basic and nonpolar amino acids such as arginine, phenylalanine, leucine, isoleucine, and valine was also high, this being followed by the MRPs from the acidic amino acids such as aspartic acid and glutamic acid, while that of MRPs from cysteine had the lowest value. As the heating time was increased, the color intensity of the MRP from lysine became 2–3 times higher than that from the other amino acids (Fig. 2).

Figure 3 shows the remaining percentages of unreacted glucose in nine MRPs during the reaction period. Glucose was gradually degraded during the course of heating, being almost completely destroyed in the MRPs from lysine and arginine after 12 h, however the MRPs from aspartic acid, glutamic acid, and cysteine respectively remained 27.25%, 16.21%, and 18% of the original glucose.

Figure 4 shows the rate of change in the color intensity of the MRPs in the presence of metal ions. After being
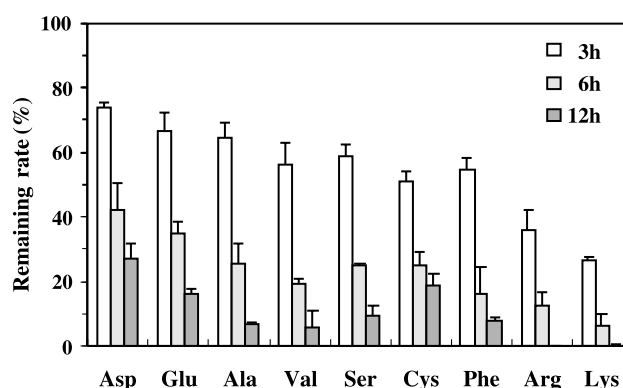

Fig. 3. Remaining percentages of glucose in the model MRPs during a reaction time of 3–12 h. Vertical bars indicate the standard deviation of the mean (n = 2)
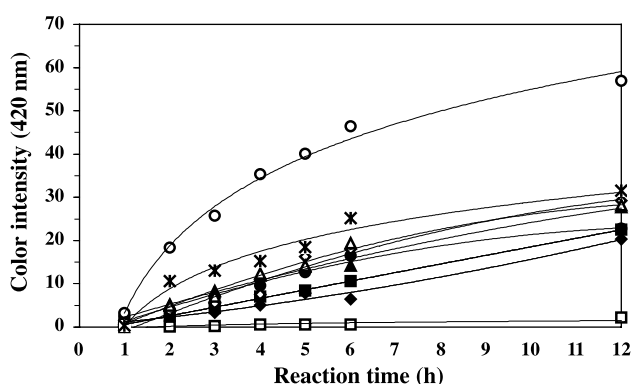

Fig. 2. Color intensity of model MRPs prepared by heating glucose with each of nine amino acids at 100°C for 1–12 h. ◆, Asp; ■, Glu; ▲, Ala; ●, Val; ◇, Ser; □, Cys; △, Phe; ✖, Arg; ○, Lys. Each value is the mean of two determinations

heated for 3 h, the rate for cysteine showed the highest value, being respectively increased by 80% and 90% by the Fe^{2+} and Cu^{2+} ions. The rates for valine and phenylalanine were also greatly enhanced, and those for most amino acids were increased by 10–40% depending on metal ion. The rate of change in color intensity of arginine was inhibited by 5–15%. A longer heating time of 6 h resulted in a different rate of change in the color intensity from the same MRPs heated for 3 h. The rates for the acidic amino acids were increased, those for the nonpolar amino acids such as valine, serine, cysteine, and phenylalanine were greatly decreased, while those for the basic amino acids remained unchanged.

In contrast, the addition of NaCl tended to inhibit the browning of MRPs heated for 6 h (Fig. 5). With 1% NaCl added, the color intensity of the MRPs from aspartic acid, glutamic acid, valine, and cysteine was inhibited by 15–20%, whereas the intensity for alanine, phenylalanine, and lysine was more enhanced than that of the control.
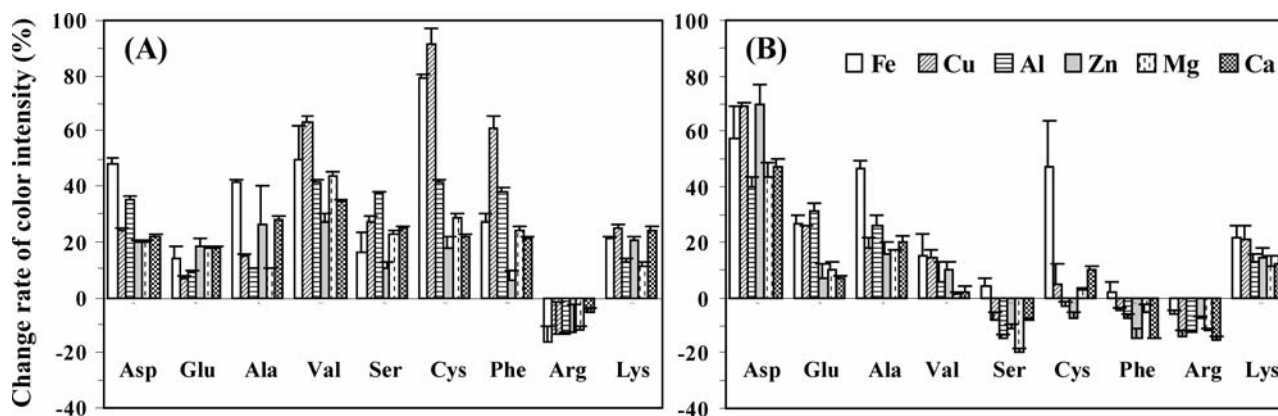
Fig. 4. Effect of metal ions on the color intensity of model MRPs heated for 3 h and 6 h. Vertical bars indicate the standard deviation of the mean (n = 2): (**A**) 3 h heating; (**B**) 6 h heating
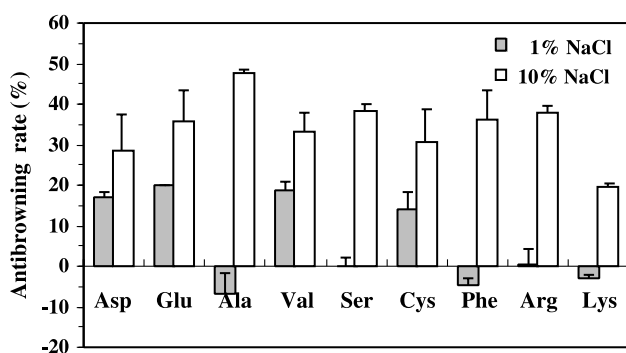

Fig. 5. Effect of NaCl on the color intensity of the model MRPs. Vertical bars indicate the standard deviation of the mean (n = 2)

However, when 10% NaCl was added, the color intensity was significantly inhibited by more than 30% in all the MRPs, with that from alanine being particularly inhibited by 47%. The lowest inhibition was 20% in the MRP from lysine.

Discussion

In order to minimize the pH decrease during the Maillard reaction, the model MRPs were each prepared in a strongly-buffered solution (Kwak et al., in press). The pH values of the 60 MRPs were maintained in the range from 6.52 to 6.10 after 12 h of heating, the values for the MRPs from the basic amino acids being generally higher than those from the acidic amino acids. There was, however, no difference in the pH value of the MRPs according to the type of sugar.

The degree of contribution to browning of the five sugars was investigated by determining the color intensity of each of the 60 MRPs heated for 3 h. The MRPs from glucose showed a higher degree of browning than those

from fructose (Hayashi and Namiki, 1986), this being ascribed to the keto sugar proceeding through imine intermediates that favor the formation of Heyns products, while the aldose sugars proceeded through Amadori products (Hodge, 1953). The rate of browning of Heyns products is known to be slower than that of Amadori products (Pilková et al., 1990). It was difficult to compare the degree of browning between the monosaccharide and disaccharide (Ashoor and Zent, 1984; Hollnagel and Kroh, 1998; Morales and Jiménez-Pérez, 2001), since this differed according to the experimental conditions. However, the results obtained are consistent with those of Kato et al. (1968) and Hayashi and Namiki (1986) that xylose, which is pentose showed much higher reactivity than glucose and fructose that are hexoses.

The color intensity of MRPs of lysine was reactivity was 2–3 times higher than that from the other amino acids. The high reactivity of lysine is attributed to the two α- and ε-amino groups (Miller et al., 1984), the result being in accordance with those of Ashoor and Zent (1984) and Ajandouz and Puigserver (1999). However, this somewhat different from the result of Adrian (1974), in which the reactivity of lysine was 5–15 times higher than that of the other amino acids. Cysteine was found to have the lowest contributory effect to browning. It is known that sulfur amino acids and peptides such as cysteine and glutathione are generally effective for inhibiting nonenzymatic browning.

Glucose and glutamic acid are the most abundant sugar and amino acid in soybean paste (Kim et al., 1990; Kaneko et al., 1994; Park et al., 1994). Soybean paste contains 6.19 g/100 g of glucose, this being 5 and 8 times more than fructose (1.20 g/100 g) and maltose (0.76 g/100 g), respectively (Kaneko et al., 1994). The

glutamic acid contained in soybean paste is 725.01 mg/100 g (Kaneko et al., 1994), which is 1.5–8 times more than that of the other amino acids, although lysine, with significant reactivity, has a small content of 287 mg/100 g (Kim et al., 1990; Park et al., 1994). Thus, if the melanoidins formed between sugar and amino acid are considered to be the only factor in the browning of soybean paste, it is thought that browning would be mostly ascribable to the melanoidins formed by glucose-glutamic acid and glucose-lysine. Although a synergistic effect from the possible interaction between amino acids could exist, this was not considered in this study, so further work is needed to investigate this possibility. It is believed that lysine could be the amino acid most strongly attributable to the browning of soybean paste during storage for a long period.

The loss in glucose of the MRPs was not essentially proportional to color intensity. Glucose loss in the MRPs from cysteine was found to be great unexpectedly high, considering their low color intensity (Fig. 2). This is ascribed to the side-reactions occurring between the amino acid and sugars and the regeneration of amino acid from the Amadori product (Yaylayan and Huyghues-Despointes, 1994). Moreover, the amino acids in the MRPs were also gradually degraded with heating time, the remaining percentages from lysine and arginine being the lowest. However, the highest percentage was not in the MRPs from cysteine, but in those from aspartic acid (data not shown). This result of amino acid loss in the MRPs was also not consistent with the color intensity.

It seems necessary to add metal ions and NaCl to soybean paste during the manufacturing process. Metal ions are likely to be contaminants in soybean paste due to the use of water containing a number of metal ions (Hatto, 1993), while NaCl is an essential component for preservation and taste. The final experiment was therefore carried out to investigate whether these components could affect browning. The effect of metal ions on browning was found to depend on the type of amino acid and heating time, as well as on the type of metal ion. Fe^{2+} and Cu^{2+} ions enhanced browning the most, with Ca^{2+} ion having the least effect. It is known that a transition metal ion catalyzes the Maillard reaction by the oxidative pathway (Morita and Kashimura, 1991). However, in this study, while the browning of most MRPs tended to be accelerated in the presence of metal ions, it was inhibited in some MRPs. Gomyo and Horikoshi (1976) have reported that browning was suppressed by the coagulation of melanoidin in the presence of various metal ions. According to Fallico and Ames (1999) and Pokorný et al. (1988), there was respectively only a small effect of iron on the model systems. Although metal ions had the greatest effect on browning the MRPs from cysteine, this effect might have been insignificant because of the low reactivity of cysteine (Ashoor and Zent, 1984).

In contrast, NaCl did inhibit browning of the MRPs. This fact indicates that the color development of soybean paste containing a high content of salt would be retarded more than that of soybean paste with a low content.

Conclusions

The browning of soybean paste seems to be largely to the melanoidins formed by glucose-glutamic acid and glucose-lysine from the result of the color intensity of 60 MRPs prepared by heating each of five sugars with each of 12 amino acids, the reactivity to browning, and the contents of sugar and amino acid in the soybean paste. Browning also tended to be accelerated in the presence of a metal ion and inhibited by NaCl. Therefore, in order to inhibit browning, we need to particularly control the browning reaction between glucose-glutamic acid and glucose-lysine in the presence of metal ion.

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