行政院國家科學委員會專題研究計畫　成果報告

以葡萄糖-甘胺酸模式探討乙醇溶液之梅納反應

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ABSTRACT

The rate of Maillard browning in ethanolic solutions of glucose－glycine increased with an increase in ethanol concentration. The rate of browning in 0－50% ethanolic solutions of glucose－glycine is higher at pH 5.5 than at 4.3. The descending order in browning potential in 50% ethanolic solution of sugar－glycine buffered at pH 4.3 is xylose, galactose, glucose, fructose, lactulose and sucrose among sugars; and that in 50% ethanolic solution of glucose－amino acid at pH 4.3 is lysine, threonine, serine, glycine, cysteine and alanine among amino acids. Ethanol inhibits hydroxymethylfurfural (HMF) formation in glucose solution. Glycine accelerates HMF formation and browning in glucose－glycine solution. The acceleration effect is enahnced by ethanol.

Key words: Maillard, browning, ethanol, hydroxymethylfurfural.

INTRODUCTION

Maillard reaction is a class of nonenzymatic browning that involves the interaction of proteins and amines with carbohydrates (Whistler and Daniel 1985). The factors that influence the rate of Maillard reaction in the processing and storage of food include the composition of food, the time-temperature condition, pH, water activity, oxygen tension, the presence of promoters and inhibitors, etc. (Ames 1998). Conventionally, research in Maillard reaction uses water as the solvent. Studies in non-aqueous systems are few.

In aqueous systems, the key chemical components that influence the progress of Maillard reaction directly are sugars and amino acids. Del Pilar Buera and others (1987) compared the activities of various monosaccharides and disaccharides below pH 6, and found that they are xylose, fructose, glucose, lactose, maltose, and sucrose in descending order. Brands and others (2000) investigated the Maillard reaction in sugar—casein system and reported that those sugars having a higher proportion of ring forms in the solution browned more rapidly. They also found that ketoses brown faster than their corresponding aldoses, and that non-reducing disaccharides brown even slower as they need to be hydrolyzed to reducing sugars first. Ashoor and Zent (1984) compared the rate of Maillard browning among common amino acids, and grouped them into high, intermediate and low browning producing groups. Higher temperature and longer time result in more serious browning (Lee and Nagy 1988). In alkaline condition, both of the browning rates in fructose—glycine and glucose—glycine systems reach maximum at pH 10 (Ashoor and Zent 1984). In the pH range between 3.4 and 7.7, the browning in starch—glucose—lysine mixture increases with the increase in pH (Bates 1994). Renn and Sathe (1997) showed the browning rate between L-leucine and D-glucose was positively correlated with pH.

The above-reviewed studies were all done in aqueous systems to stand for common foods. However, some foods, such as beer, sake, rice wine, many fortified wines and liqueurs are sorts of ethanolic solutions that contain a noticeable amount of Maillard reaction substrates. The investigation of Maillard browning in ethanolic solution shall be worthwhile.

MATERIALS AND METHODS

Glucose (0.2 M)—glycine (0.2 M) model solutions were prepared by dissolving glucose and glycine in 0.05 M succinic acid—sodium hydroxide buffered 0—50% ethanolic solutions. The pH value was adjusted to 4.3 to stand for sake, or 5.5 to stand

for a liqueur made from spirit and a low acid food. Aliquots of the model solutions were transferred to vials, hermetically caped, and heated in a water bath at 100 ℃ for 0－6 hr.

Xylose, fructose, galactose, sucrose, lactose or lactulose was then used in substitution for glucose in the preparation of model solutions containing 0%, 15%, and 50% ethanol to investigate the browning potential of different sugars in ethanolic system. Similarly, lysine, threonine, cysteine, serine or alanine was used in substitution for glycine in the preparation of ethanolic model solutions for investigating the effect of amino acid species on the Maillard browning. The 0%, 15%, and 50% ethanol concentrations were chosen to stand for the ethanol concentrations in aqueous solution, sake, and liqueur, respectively.

The absorbance at 420 nm was taken as a measure of the extent of browning (UV/VIS 8500 Double-Beam Spectrophotometer, Lab Alliance, State College, PA). The content of hydroxymethylfurfural (HMF) was analyzed adapting the method reported by Lee and Nagy (1988). The model solutions were incubated at 100℃ for 6 hr, and then injected into an HPLC system that was composed of a Luna 5 ì C18(2) Column (Phenomenex, Torrance, CA), a Series Ⅲ Pump (Lab Alliance), a UV－970 Intelligent UV/VIS Detector (Jasco Co., Tokyo, Japan). The mobile phase was acetonitrile－water－acetic acid in 10:89.5:0.5 volume ratio at a flow rate of 1.0 ml/min. The detected wavelength was 280 nm.

RESULTS AND DISCUSSION

Effect of ethanol concentration on pH and browning in non-buffered solutions

The effect of ethanol concentration on the pH value and the absorbance at 420 nm of non-buffered glucose (0.2 M)－glycine (0.2 M) solutions is shown in Figure 1. In the range of 0－50% ethanol content, a higher ethanol content corresponds to a higher initial pH value (Figure 1a). Similar phenomenon has been reported in reconstituted wines in 0－22% ethanol content (Gutiérrez 2003). The pH in the ethanolic solutions decreased during incubation, similar to the findings in aqueous glucose－glycine solutions reported by Morales and Jimenez-Perez (2001). Among all the samples incubated for the same duration, the one containing no ethanol showed the lowest pH value. The decrease in pH observed during the Maillard reaction could be attributed to the degradation of sugar into acid (Beck and others 1990), or the condensation between the free amino group of amino acid and the carbonyl group of glucose (Trifiro and others 1990).

Figure 1b shows that the period of rapid increase in absorbance in the

non-buffered solutions was preceded by an “induction” period when little browning occurred. Similar results were found in aqueous solutions of glucose－glycine (Ajandouz and Puigserver 1999), glucose－fructose－glycine (Mundt and Wedzicha 2003), xylose－proline (Peterson and others 1994), and glucose－essential amino acids other then glycine (Ajandouz and Puigserver 1999). The sample containing 50% ethanol had the highest browning rate among all the samples tested.

Effect of pH on browning in ethanolic solutions

The effect of ethanol concentration on the absorbance at 420 nm in glucose (0.2 M)－glycine (0.2 M) solutions buffered at pH 4.3 and pH 5.5 by 0.05 M succinic acid －sodium hydroxide is shown in Figure 2.

At pH 4.3, the browning rate increased with an increase in ethanol concentration after the latter reached approximately 20% (Figure 2a). For example, after incubating at 100 ℃ for 6 hr, the absorbance at 420 nm in the 50% ethanolic solution reached 1.03, roughly 3.8－3.6 times the absorbance value of 0.27－0.29 in the 0－20% ethanolic solutions.

In all the pH 5.5 samples, the browning rate increased with the increase in ethanol concentration (Figure 2b). Control samples that contained 0.2 M glucose only were incubated at 100 ℃ for 6 hr in a separate experiment. The absorbances at 420 nm of these control samples were found to stay near 0 (data not shown). It means that the contribution to browning from caramelization can be neglected in the present treatments. Generally, severe heating intensity and extreme pH are necessary for the caramelization of sugar in aqueous solutions (Morales and Van Boekel 1998). The same thing could be true in ethanolic solutions as well.

The value of pH influences the relative importance among various pathways in Maillard browning, and therefore influences the profile of reaction products and the extent of browning (Ames 1998). The comparison in browning rate among the incubated ethanolic solutions of glucose (0.2 M)－glycine (0.2 M) buffered at pH 4.3 and pH 5.5 can be seen in Figure 3. Browning rate increases with the increase in pH from 4.3 to 5.5. Restated, a low pH value inhibits the progress of Maillard browning in ethanolic solutions, similar to the reported findings in aqueous solutions (Petriella and others 1985, Del Pilar Buera and others 1987). Figure 3 also shows that no matter the solution is buffered or not, it browns much more rapidly at a higher ethanol concentration.

Effect of sugar species on browning in ethanolic solutions

The sugar species tested in the present study covered pentose, hexose, monosaccharide, disaccharide, aldose, and ketose.

Table 1 presents the absorbance at 420 nm in 0%, 15% and 50% ethanolic solutions of glycine (0.2 M) and a sugar (0.2 M) buffered at pH 4.3 with 0.05 M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hr. In solutions containing no ethanol, the browning rates of xylose, fructose, galactose, lactulose, glucose, sucrose, and lactose are one after another in descending order. Among them, xylose as a pentose has the highest browning rate; fructose and lactulose as ketoses brown faster than glucose and lactose, the corresponding isomeric aldoses, respectively; and disaccharides including lactose and sucrose brown slowest, probably because they need to be hydrolyzed before participating in the Maillard reaction (Brands and others 2000). However, in 50% ethanolic solutions, the descending order for browning rates is changed to xylose, galactose, glucose, fructose, lactulose, and sucrose. The data for lactose are not available due to the low solubility of this sugar in the solution. Although a pentose still browns fastest and disaccharides brown slowest, an aldose such as glucose may become faster than its ketose isomer, fructose in this example. It appears that the concentration of ethanol may influence the reactivity of some, but not all, of the sugar species.

Effect of amino acid species on browning in ethanolic solutions

The amino acids tested beside glycine were lysine, alanine, serine, threonine and cysteine. Table 2 shows the browning in ethanolic solutions of glucose (0.2 M) and an amino acid (0.2 M) buffered at pH 4.3 and incubated at 100 ℃ for 6 hr. Among all the samples, the most serious browning occurred in glucose－lysine solution. It is well recognized that the carbonyl group of glucose condenses most easily with the ε -amino group of lysine in aqueous solutions to form N-substituted glycosylamine in the initiation stage of Maillard browning (Van Martins and others 2001). The same thing would be true in ethanolic solutions.

Table 2 also shows that, the browning rate in all the glucose－amino acid combinations increased with an increase in ethanol concentration from 15% to 50%. For example, the absorbance readings at 420 nm were 5.73 and 15.94 in the incubated 15% and 50% ethanolic solutions of glucose－lysine mixture respectively.

In solutions containing no ethanol, the browning rates of lysine, threonine, serine, glycine, alanine, and cysteine were found to be one after another in descending order. These amino acids except threonine followed the same order as reported by Ashoor and Zent (1984) in another aqueous system at pH 9.0. The difference in the behavior of threonine may be resulted from the difference in pH. Threonine at a lower pH, which is 4.3 in the present study, stands on a higher position among amino acids in the browning rate.

In 50% ethanolic solutions, the order for browning rates is changed to be lysine,

theronine, serine, glycine, cysteine, and alanine, one after another.

Formation of HMF in ethanolic solutions

Figure 4 shows the absorption spectra in the region 200-700nm for glucose (0.2 M) and glucose (0.2 M)－glycine (0.2 M) solutions that were buffered at pH 4.3 with 0.05 M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hr. An absorption peak occurred at 280-290nm.

The maximum absorbance in the spectrum of HMF occurs at 280nm (Lee and Nagy 1988). Therefore, we recognize the above-mentioned absorption peak 280-290nm as an evidence for HMF formation. It was later reconfirmed by HPLC analysis. The retention times for this peak in model solutions and in the standard HMF solution were found to be the same.

Figure 4 shows the relation between ethanol concentration and HMF content in glucose solution (0.2 M) (Figure 4a) and glucose (0.2 M)－glycine (0.2 M) solution (Figure 4b) that were buffered at pH 4.3 with 0.05 M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hr. A higher ethanol concentration corresponds to a lower HMF content in the glucose solution, and higher HMF content in the glucose－glycine solution. Restated, ethanol inhibits HMF formation in glucose solution, while glycine accelerates HMF formation in glucose－glycine solution and the acceleration effect increases with the increase in ethanol concentration.

Figure 3 and Figure 4b together suggest that ethanol accelerates the Maillard browning through the formation of more HMF in glucose－glycine solution.

However, ethanol does not promote browning in fructose－glycine and lactulose －glycine solutions (Table 1). We suspect there are mechanisms other than the hydrophilicity for ethanol to effect on the rate of Maillard browning.

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Table 1—Absorbance at 420nm of the ethanolic solutions of glycine (0.2 M)－sugar (0.2 M) buffered at pH 4.3 with 0.05 M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hours

Ethanol concertration	Glucose	Fructose	Lactose	Lactulose	Sucrose	Galactose	Xylose
0%	$0.26\pm0.01^{e*y**}$	1.14 ± 0.01^{bx}	0.23 ± 0.01^{ex}	0.56 ± 0.01^{dx}	0.25 ± 0.03^{ey}	0.75 ± 0.02^{cy}	6.28 ± 0.08^{az}
15%	0.25 ± 0.01^{dy}	0.86 ± 0.01^{by}	0.17 ± 0.01^{dy}	0.47 ± 0.01^{cy}	0.20 ± 0.01^{dz}	0.77 ± 0.01^{by}	10.94 ± 0.28^{ay}
50%	1.29 ± 0.02^{bx}	1.14 ± 0.01^{bx}	---***	0.49 ± 0.02^{cy}	0.43 ± 0.02^{cx}	0.97 ± 0.07^{bx}	33.51 ± 2.04^{ax}

*: $^{a-e}$Different letters within the same row are significantly different ($p<0.05$) in Duncan's multiple range test.

**: $^{x-z}$Different letters within the same column are significantly different ($p<0.05$) in Duncan's multiple range test.

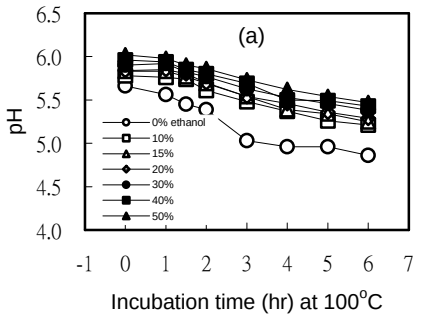
***: Insoluble in 50% (v / v) ethanol.

Table 2—Absorbance at 420nm of the ethanolic solutions of glucose (0.2 M)－amino acid (0.2 M) buffered at pH 4.3 with 0.05M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hours

Ethanol concentration	Alanine	Cysteine	Glycine	Serine	Threonine	Lysine
0%	$0.10\pm0.01^{c*y**}$	0.08 ± 0.01^{cz}	0.24 ± 0.02^{cy}	0.28 ± 0.04^{cy}	0.64 ± 0.02^{bz}	3.23 ± 0.40^{az}
15%	0.07 ± 0.01^{cy}	0.15 ± 0.02^{cy}	0.22 ± 0.02^{bcy}	0.27 ± 0.01^{bcy}	0.79 ± 0.02^{by}	5.73 ± 0.79^{ay}
50%	0.32 ± 0.04^{cx}	0.59 ± 0.05^{cx}	1.00 ± 0.02^{cx}	1.03 ± 0.05^{cx}	2.52 ± 0.09^{bx}	15.94 ± 0.42^{ax}

*: $^{a-e}$Different letters within the same row are significantly different ($p<0.05$) in Duncan's multiple range test.

**: $^{x-z}$Different letters within the same column are significantly different ($p<0.05$) in Duncan's multiple range test.



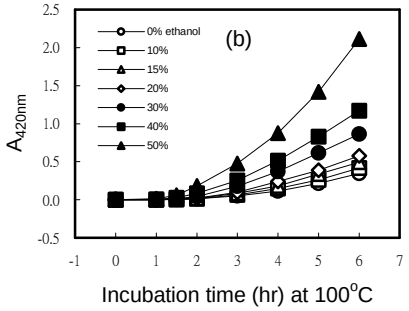
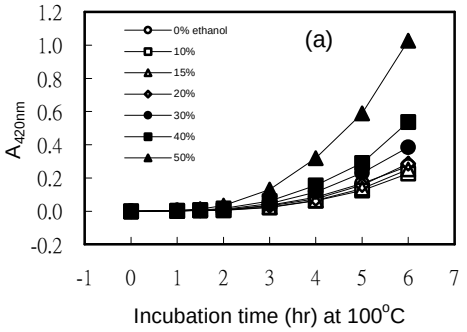


Figure 1—Effect of ethanol concentration on (a) the pH and (b) the absorbance at 420nm in glucose (0.2 M)－glycine (0.2 M) solutions.



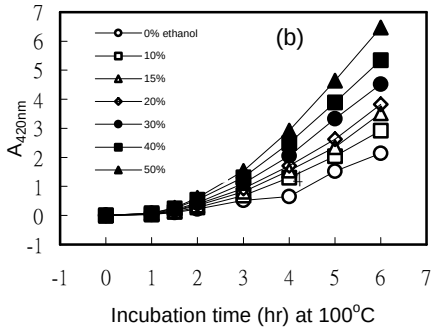


Figure 2—Effect of ethanol concentration on the absorbance at 420nm in glucose (0.2 M)－glycine (0.2 M) solutions buffered at (a) pH 4.3 and (b) pH 5.5 by 0.05 M succinic acid－sodium hydroxide.

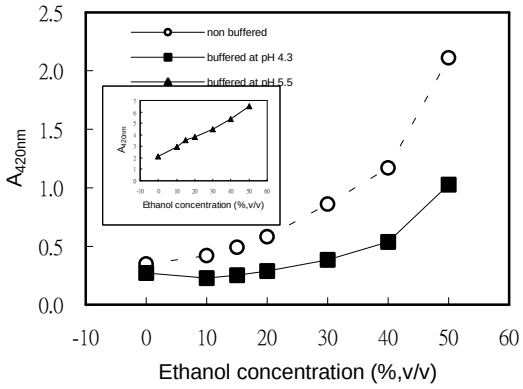


Figure 3—Absorbance at 420nm of the ethanolic solutions of glucose (0.2 M)－glycine (0.2 M) after incubating at 100 ℃ for 6 hours.

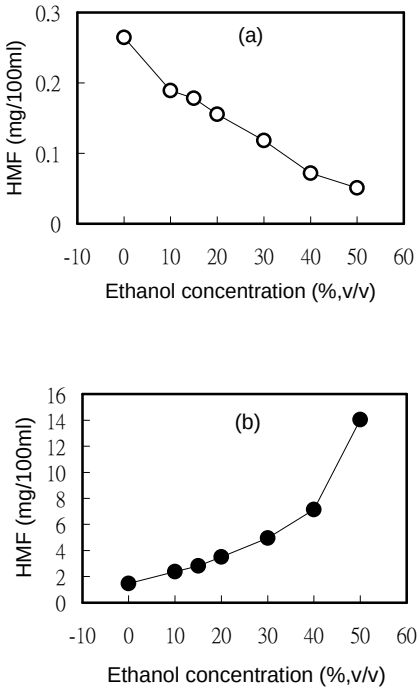


Figure.4—The relation between ethanol concentration and HMF content in (a) glucose (0.2 M) and (b) glucose (0.2 M)－glycine (0.2 M) solutions buffered at pH 4.3 with 0.05 M succinic acid－sodium hydroxide and incubated at 100 ℃ for 6 hours.

計劃成果自評：

本計劃第一年之研究進度與原計劃相符，並完滿達成預期之目標，茲將最重要發現簡述如下：

1. 酒精濃度對梅納褐變產物之影響：確認乙醇會加速 glucose-glycine 模式系統反應液梅納反應之速率。
2. 反應因子對酒精溶液中梅納褐變產物之影響：當乙醇濃度提高至 50%時，褐變反應之速率以五碳糖為最高，雙糖最低，此與水溶液相似，但是醛糖之反應速率反而高於酮糖；對胺基酸與葡萄糖之梅納褐變反應而言，褐變反應速率依序為 Lysine＞Thr＞Gly＞Ser＞Cys＞Ala。
3. 含硫氫基化合物抑制酒精溶液中梅納反應之探討：某些硫醇類化合物可有效抑制 glucose-glycine 之梅納反應，例如添加 0.01M 之 L-Cysteine, N-acetyl-L-cysteine, glutathione 或 sodium bisulfite 時，抑制效果均超過 50%。
4. 部分研究成果已投稿於 Journal of Food Science。