Food Sci. Biotechnol. Vol. 17, No. 5, pp. 931 ~ 939 (2008)

Food Science and Biotechnology

Effect of pH on the Enolization of Sugars and Antioxidant Activity of Caramelization Products Obtained by Caramelization Browning

Ji-Sang Kim and Young-Soon Lee*

Department of Food and Nutrition, Kyung Hee University, Seoul 130-170, Korea

Abstract The objective of this study was to investigate the enolization reaction and the antioxidant activity of caramelization products (CPs) obtained by caramelization browning of glucose and fructose solutions prepared at a pH ranging from 7.0 to 12.0 at varying temperatures (80-180°C). The degradation of sugars rapidly increased at a high alkaline pH (10.0-12.0), and fructose degraded more rapidly than glucose ($p<0.05$). As the pH increased, the degree of sugar enolization was higher in fructose than in glucose. Browning and the formation of intermediate degradation products increased with the increase in heating temperatures. The browning development was dependent upon the type of sugar, and it was generally higher at alkaline pH than at neutral pH. The reducing power and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the CPs increased with the increase in browning and formation of large amounts of intermediates. Therefore, the CPs with pronounced antioxidant activity can be prepared by heating fructose or glucose solutions that have a very alkaline pH to high temperatures.

Keywords: antioxidant activity, caramelization, enolization

Introduction

Non-enzymatic browning reactions occurring in food during processing and storage has known to contribute to food quality and acceptability. Those reactions, including the Maillard reaction, caramelization, chemical oxidation of phenols and maderization are favored by heat treatments (1).

Among non-enzymatic browning reactions, the Maillard reaction has been the most intensively studied. Maillard reaction products (MRPs) have been found to exhibit antioxidantactivity due to radical scavenging activity (2,3), metal chelating activity (4), scavenging of active oxygen species (5), as well as decomposition of hydroperoxide (6). During the development of brown color caused by the Maillard reaction, caramelization can occur simultaneously (7). Caramelization reactions contribute to overall nonenzymatic browning, especially in the alkaline pH ranges, leading to an overestimation of the Maillard reaction in foods (8,9). Caramelization involves the heat-induced decomposition of sugar, normally monosaccharides (10). Monosaccharides in aqueous alkaline solutions undergo both reversible and irreversible transformations (11). The reversible reaction includes ionization, resulting in equilibrium of neutral and ionized monosaccharides; mutarotation, resulting in equilibrium of the different cyclic hemiacetal structures of monosaccharides; and enolization, resulting in the transformation of interconvertible monosaccharides (12).

Enolization reaction known as the 'Lobry de Bruyn-Alberda van Ekenstein rearrangement' produces enediol anion species. A subsequent chain of irreversible reactions, known as the alkaline degradation reaction, leads to the formation of organic acid products. Aldolization and retroaldolization of carbonyl compounds result in an elongation and fragmentation of the carbon chain, respectively.

The products of alkaline degradation reaction with high molecular weight are associated with color formation (13). Caramelization reaction partially contributed to the browning of Maillard reaction (8,9). Very little attention has been paid so far to the contribution of caramelization to the nonenzymatic browning reactions of glucose or fructose, although studying the chemical reactions involved in caramelization is a prerequisite for understanding the Maillard reaction. It has, however, been clearly established that the fragmentation of sugars occurs to a little extent at a low pH levels (7,14) and increases considerably at a high pH levels and temperatures, yielding colored *N*-free polymers (15,16). The caramelization products (CPs) was reported to have antioxidant activities (17). Acetone extracts from glucose CPs reduced peroxide values of soybean oil (18). The CPs from fructose could inhibit lipid oxidation in saury mince effectively during iced storage (17). The CPs from different sugars showed different reducing powers and radical scavenging activities. Generally, the CPs prepared in alkaline solutions exhibited greater antioxidant activity than those obtained under neutral pH. The CPs from hexose also had superior antioxidant activity to those from pentose (17). However, few studies have been conducted to compare the antioxidant activities of the CPs by the enolization reaction of sugars. Therefore, the CPs with high antioxidant activity could be prepared using the appropriate sugar and pH condition. The objective of this study was to investigate enolization reaction of sugars and antioxidant activity of the CPs in caramelization browning from glucose and fructose solutions prepared at a pH ranging from 7.0 to 12.0 with heating for various temperatures.

*Corresponding author: Tel: +82-2-961-0881; Fax: +82-2-968-0260
E-mail: yysllee@khu.ac.kr
Received October 11, 2007; Revised March 30, 2008;
Accepted March 31, 2008

Materials and Methods

Chemicals D-Glucose, D-fructose, ferric chloride, ferrous chloride, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Trichloro-acetic acid (TCA), potassium ferricyanide, sodium carbonate, and sodium hydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany).

Preparation of CPs D-Glucose and D-fructose were dissolved in 1 M sodium phosphate buffer, pH 7.0-9.0 or 1 M sodium carbonate buffer, pH 10.0-12.0 to obtain a final concentration of 1 M. Ten mL of each sugar solution were transferred to a screw-capped test tube and subjected to heating in a silicone oil bath at various temperatures (80 to 180°C). At the heating temperatures designated, the samples were taken and cooled in iced water immediately. The samples were stored at 4°C until used for analysis and performed within 48 hr.

Determination of reducing sugar and enolization reaction The reducing sugar content and enolization reaction of the CPs were determined using an HP 1100 liquid chromatography (Hewlett Packard, Wilmington, DE, USA). An Agilent quaternary pump connected to an reflectance index (RI) detector (Model G1362A; Hewlett Packard) was used with a zorbax carbohydrate column (4.6×250 mm, 5 μm particle size, Agilent Technologies, Wilmington, DE, USA). The mobile phase of acetonitrile:water (75:25, v/v) was delivered at a flow rate of 2.0 mL/min. The column temperature was 30°C and 1 μL portion of what was injected into the high performance liquid chromatography (HPLC) system. The data analysis was performed using Chemstation software (Hewlett Packard). The changes in reducing sugar content were expressed as the relative concentration (%) in comparison with the original content.

Ultraviolet (UV) absorbance and browning determination The formation of intermediate products was monitored by their absorbance at peak values. UV absorbance was measured at wavelengths of 270 and 285 nm using a UV-VIS spectrophotometer (UV 160A; Shimadzu Co., Kyoto, Japan), as described by Benjakul *et al.* (17). Prior to UV absorbance determination, samples prepared at pH ranging from 10.0 to 12.0 were diluted to 100-500 times using the distilled water. Browning intensity was determined by monitoring the absorbance at 420 nm. Two-hundred fold dilution was made for samples prepared at pH ranging from 10.0 to 12.0. The absorbance ratio (A_{270}/A_{420} and A_{285}/A_{420}) was also calculated to monitor the transformation of UV-absorbing compounds into brown polymers.

Determination of DPPH radical scavenging activity DPPH radical scavenging activity was determined according to the method of Yen and Hsieh (3), with a slight modification. Two-hundred fold dilution was made for all

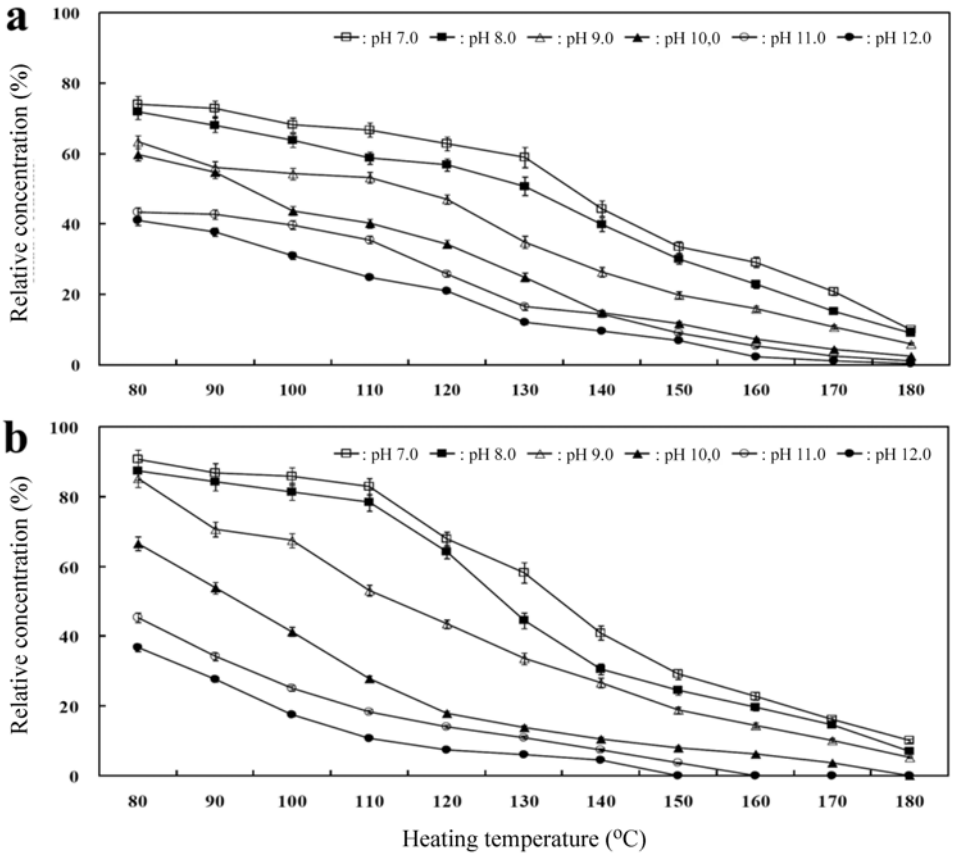

Fig. 1. Change in the reducing sugar content of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

samples before analysis using the distilled water as the diluents. A 400 μL of the CPs solution were added with 2 mL of DPPH solution (0.12 mM in 95% methanol). The reaction mixture was mixed well and incubated at room temperature for 30 min in the dark. The absorbance of the resulting solution was read at 517 nm against the blank. The blank was prepared in the same manner except that the buffer was added instead of the CPs. The radical scavenging activity was measured as a decrease in the absorbance of DPPH-sample mixture and calculated using the following equation:

$$\text{Scavenging activity sample} = [1 - (A_{sample}/A_{blank})] \times 100$$

Where A_{sample} is A_{517} of sample and A_{blank} is A_{517} of blank.

Determination of reducing power The reducing power of the CPs was measured as described by Oyaizu (19), with a slight modification. Prior to analysis, 200-fold dilution was made for the CPs prepared at pH ranging from 7.0 to 12.0. A 0.5 mL of CPs solution was mixed with 0.5 mL of 0.2 M sodium phosphate buffer, pH 6.6 and 0.5 mL of 1%(w/v) potassium ferricyanide. The reaction mixture was incubated at 50°C for 20 min and 0.5 mL of 10%(w/v) tricarboxylic acid (TCA) was then added. Thereafter, 2 mL of mixture was added with 2 mL of distilled water and 400 μL of 0.1%(w/v) ferric chloride. The absorbance was read at 700 nm using a UV-VIS spectrophotometer. Any increase in absorbance at 700 nm indicated an increased reducing power.

Statistical analysis All experimental data were analyzed by analysis of variance (ANOVA) and significant differences among means from triplicate analysis at ($p<0.05$) were determined by Duncan's multiple range tests using the statistical analysis system (SPSS 12.0 for Windows, SPSS Inc, Chicago, IL, USA).

Results and Discussion

Degradation of sugars and enolization reaction The reducing sugar content of the CPs from glucose and fructose prepared at different pH levels is shown in Fig. 1. With the increased in heating temperature, the reducing sugar content in both the sugar solutions was found to decrease ($p<0.05$). At a high alkaline pH of 10.0 to 12.0 and a temperature of 100°C, the degradation of both sugars increased rapidly ($p<0.05$). Thereafter, a slight decrease in the reducing sugar content was observed with the increase in temperature to 180°C. At pH 7.0 and 8.0, only a slight decrease in the reducing sugar content was evident. The degradation of fructose was slightly greater than that of glucose at a pH 7.0-8.0. Both fructose and glucose degraded to a great extent at 100°C under alkaline conditions (8,9,20). Benjakul *et al.* (17) also found that sugars degraded more rapidly in alkaline solutions than in neutral solutions.

A change in the enolization reaction in the CPs from glucose and fructose prepared at different pH levels is shown in Fig. 2. Glucose was converted into fructose and

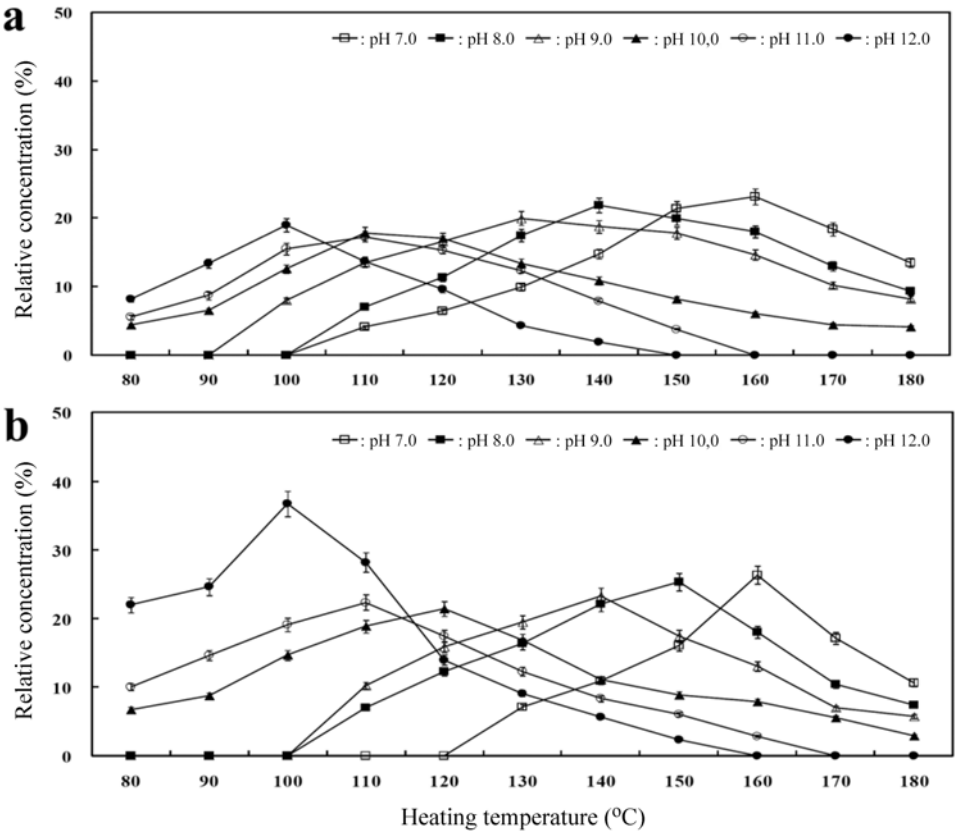

Fig. 2. Change in the enolization reaction of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

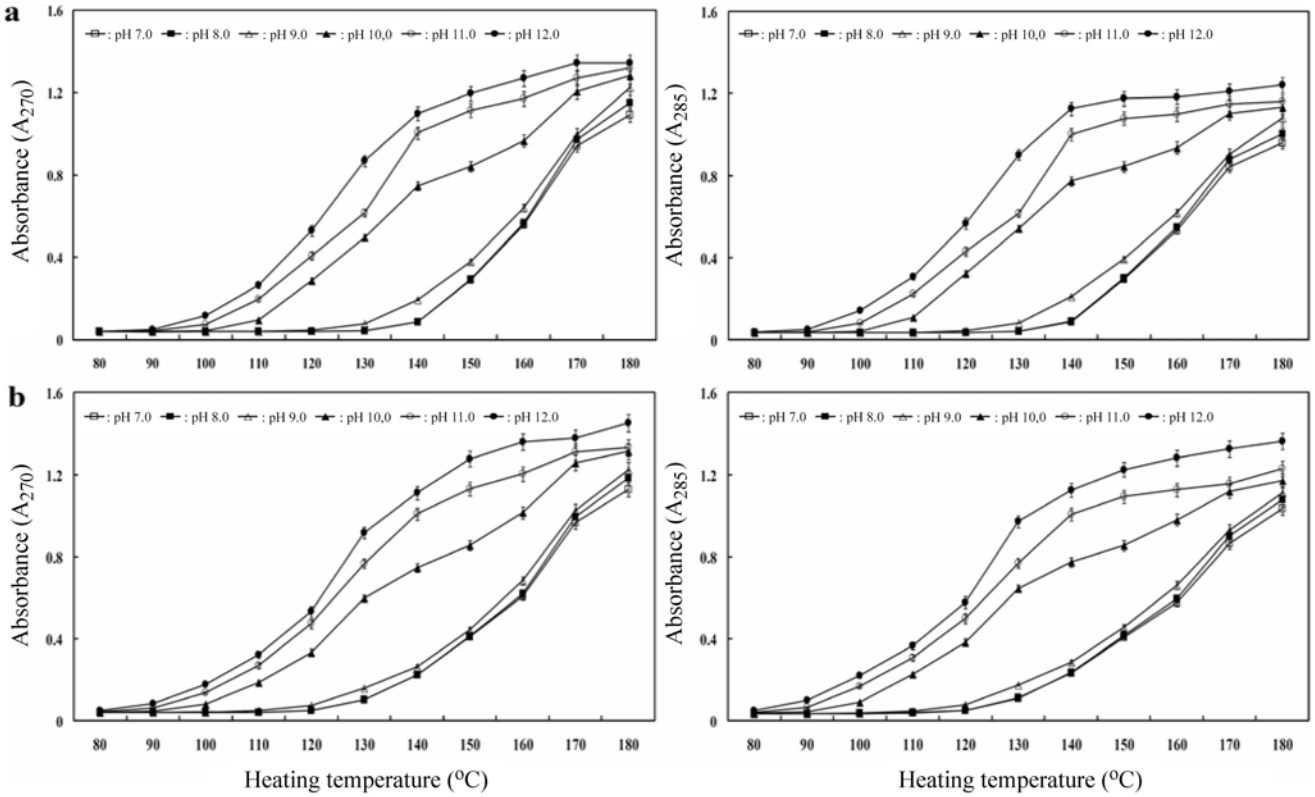

Fig. 3. Change in the UV of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

vice versa via the Lobry de Bruyn-Alberda van Ekenstein transformation. In the present study, for the enolization of both sugars, the reaction temperature of enolization was lowered with the increase pH level. The degree of enolization of the fructose solution was greater than that of the glucose solution. Enediol anions can be generated by hydroxide ions in alkaline solutions via the de Bruijn van Eckenstein rearrangement. Enediol anions are common intermediates in the isomerization reactions of monosaccharides and primary intermediates in alkaline degradation reactions (13). The enolization reaction is of particular importance because it initiates subsequent reactions that result in the degradation of aliphatic sugars (21). Our results suggest that both glucose and fructose underwent degradation to a greater extent under alkaline pH conditions. The enolization might be favored by alkaline pH condition, leading to the transformation of these sugars.

UV absorbance and browning of CPs The UV absorbance of the CPs from glucose and fructose solutions that were prepared at different pH levels is shown in Fig. 3. The UV absorbance was used to monitor the intermediate degradation products of nonenzymatic browning reactions (9,22). In addition, the absorbances at both 270 and 285 nm are measured to monitor the development of nonfluorescent intermediate products in the caramelization process (17). In the present study, for the CPs from both glucose and fructose solutions prepared at a pH ranging from 10.0 to 12.0, the absorbances at both 270 and 285 nm increased with the increase in heating temperature ($p<0.05$). The CPs from fructose showed greater absorbances at both 270 and 285 nm than those from glucose. Even at a pH of 7.0-9.0 and an increase in heating temperature to 110°C, no changes in the absorbances were observed at both 270 and 285 nm. However, the UV absorbance of the CPs at a pH ranging from 10.0 to 12.0 increased continuously with the increase in heating temperature ($p<0.05$). The results suggest that the sugars underwent degradation, and the intermediate products were formed concomitantly. Further, that the formation of intermediate products and the degradation of the sugars occurred simultaneously was evident by the decrease in the reducing sugar content (Fig. 1). This result is in agreement with those obtained by Ajandouz *et al.* (9) and Benjakul *et al.* (17), who reported that the UV absorbance of CPs increased with the increase in pH levels. Thus, large amounts of intermediate degradation products are developed at high pH levels than at low pH levels.

The browning of the CPs from both sugar solutions that were prepared at different pH levels is shown in Fig. 4. The final stage of the browning reaction was monitored by the increase the absorbance at 420 nm (9). In the present study, similar changes were observed in both sugars at 420 nm. For the CPs derived from both sugar solutions prepared at a pH ranging from 10.0 to 12.0, the browning intensity rapidly increased with the increase in heating temperature ($p<0.05$). The browning intensity of the CPs derived from fructose was greater than of the CPs derived from glucose. However, no changes in the browning intensity were

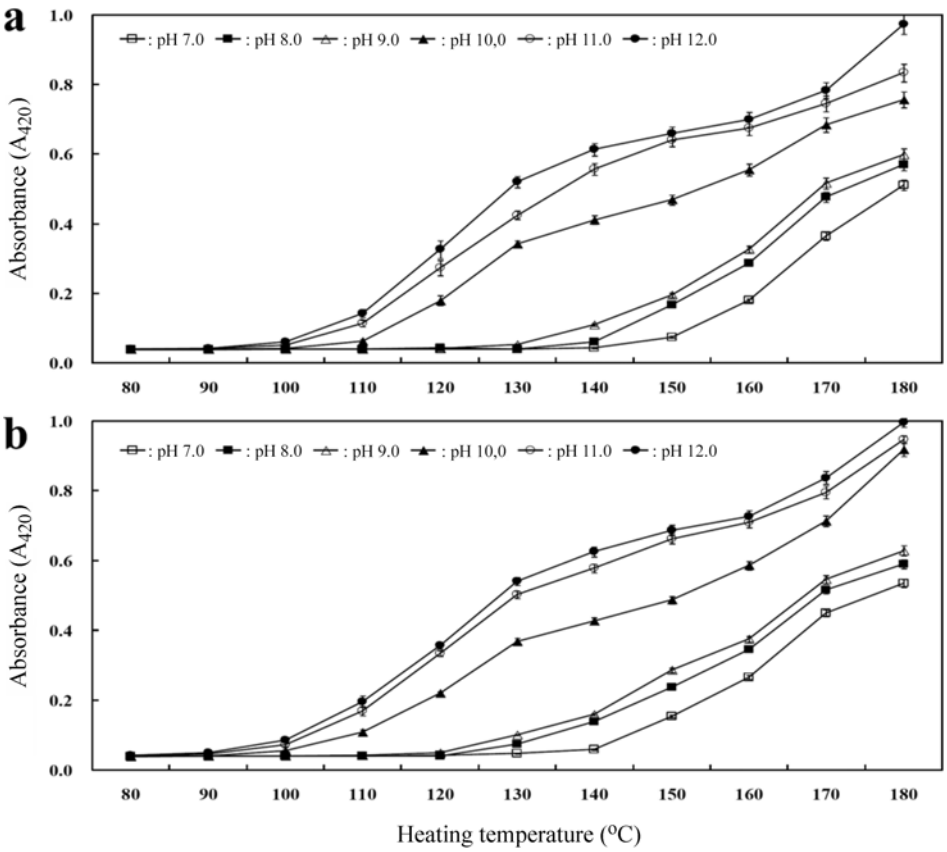

Fig. 4. Change in the browning of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

observed at a pH ranging from 7.0 to 9.0 and when the temperature was increased to 120°C. Thereafter, browning increased continuously with the increase in temperature but at a lower rate. The rate of browning development of both sugars was much lower at a pH ranging from 7.0 to 9.0 than at that a pH ranging from 10.0 to 12.0. With the increase in pH levels, the reaction temperature of browning development decreased and the rate of the browning reaction increased. The results are in accordance with those obtained by Buera *et al.* (7), Ajandouz and Puigserver (8), Ajandouz *et al.* (9), and Benjakul *et al.* (17), who reported that the browning of sugars increased with the increase in pH (alkaline). The results show that the development of browning and the formation of intermediate products occurred simultaneously (Fig. 3). The intermediate degradation products formed via enolization, known as color precursors, include methylglyoxal, glyceraldehyde, hydroxymethyl furaldehyde, furfural, and hydroxyacetylfuran (21,23,24). In addition, Coca *et al.* (13) found that the degradation products, which have less than 6 carbon atoms, can polymerize to form high molecular weight compounds, which cause the color formation during the alkaline degradation reactions. The differences in browning for both glucose and fructose might be related to their different relative structural stabilities, including mutarotation, opening of the hemiacetal ring, and enolization (25). Browning development is influenced by the type of sugar and pH levels, and the rate at which color development decreases as the pH decreases. Buera *et al.* (25) reported that the rates of browning development for reducing sugars via the caramelization processes were in the following descending order: fructose>xylose>lactose>maltose>glucose. A similar relationship between the increase in UV absorbance and browning suggested that a large proportion of the intermediate products were converted to a brown polymer (9). It is suggested that the sugars caramelized to a greater extent under alkaline conditions and that caramelization was influenced by the type of sugar. Generally, fructose is more prone to caramelization than glucose, as evidenced by its greater browning as well as the formation of large amounts of intermediates.

The absorbance ratios of glucose and fructose prepared at different pH levels are shown in Table 1 and 2. The highest A_{270}/A_{420} and A_{285}/A_{420} ratios of glucose solution were observed with a heating temperature of 150°C, whereas fructose had a maximum ratio at pH 7.0 when the heating temperature was increased to 140°C. The maximum absorbance ratio was obtained with the decrease in temperature and increase in the pH level. The increase in the absorbance ratios suggested that large amounts of intermediates were generated, and their rate of transformation to form brown polymers was low. The subsequent decrease in absorbance ratios suggested the formation of brown polymers from the intermediates. The higher absorbance ratios of the CPs obtained under neutral pH conditions indicated that large amounts of intermediates were

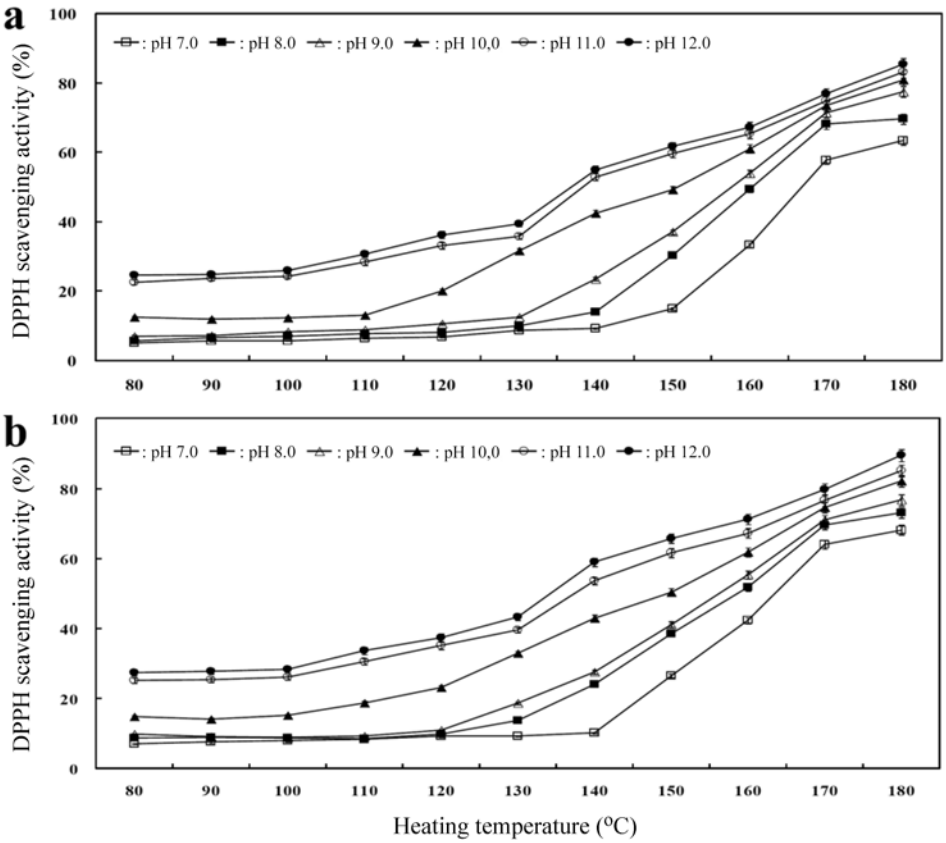

Fig. 5. Change in the DPPH scavenging activity of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

generated as compared to the final products. Similarly, the browning intensity of the CPs obtained under alkaline conditions, was greater than that the CPs obtained under neutral conditions (Fig. 4). It is suggested that the sugars are caramelized to a greater extent under alkaline conditions and that caramelization depends upon the type of sugar.

DPPH radical scavenging activity of CPs One of the chromogen radical compounds that can directly react with antioxidants is DPPH (26). DPPH changes from purple to yellow when it is scavenged by antioxidants through the donation of a hydrogen atom to form a stable DPPH-H molecule (27,28). The stable DPPH radical has been widely used for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials (29). In the present study, the DPPH radical scavenging activity of the CPs obtained from both sugars increased with the increase in heating temperature and pH levels ($p<0.05$) (Fig. 5). The results show that the DPPH radical scavenging activity was greater in the CPs obtained at higher pH levels than in those obtained at lower pH levels ($p<0.05$). The DPPH radical scavenging activity of the CPs obtained from fructose was greater than that of the CPs obtained from glucose. The higher radical scavenging activity of the CPs prepared at pH 12.0 corresponded to their higher reducing power, browning, and intermediate-product formation. A sharp increase in the DPPH radical scavenging activity was observed for both sugars at a pH ranging from 10.0 to 12.0 and an increase in heating temperature up to 100°C. However, no changes in the radical scavenging activity were observed for both sugars at a pH ranging from 7.0 to 9.0 and an increase in heating temperature up to 120°C ($p<0.05$). The activity of the CPs obtained from both sugars was somewhat different, although a similar pattern of changes was noticeable. DPPH is one of the compounds that is a proton free radical with a characteristic absorption that decreases significantly on exposure to proton radical scavengers (30). The CPs reduced the DPPH radical to a yellow compound called diphenylpicrylhydrazine (17). Either the intermediate degradation product or the brown polymer had an antioxidant activity (10). Kirigaya *et al.* (31) reported that the increase in color intensity reflects the increase in antioxidant activity. However, Rhee and Kim (18) reported that effective antioxidant compounds were formed at the early stages of browning reactions. Therefore, the CPs exhibited antioxidant activity that varied with the sugar type and the pH of the reaction. The CPs from glucose or fructose, especially those obtained from glucose and fructose solutions at high alkaline pH, were effective primary antioxidants. Furthermore, the CPs in conjunction with the MRPs may function as antioxidants in foods cooked at high temperatures, such as grilled or roasted products.

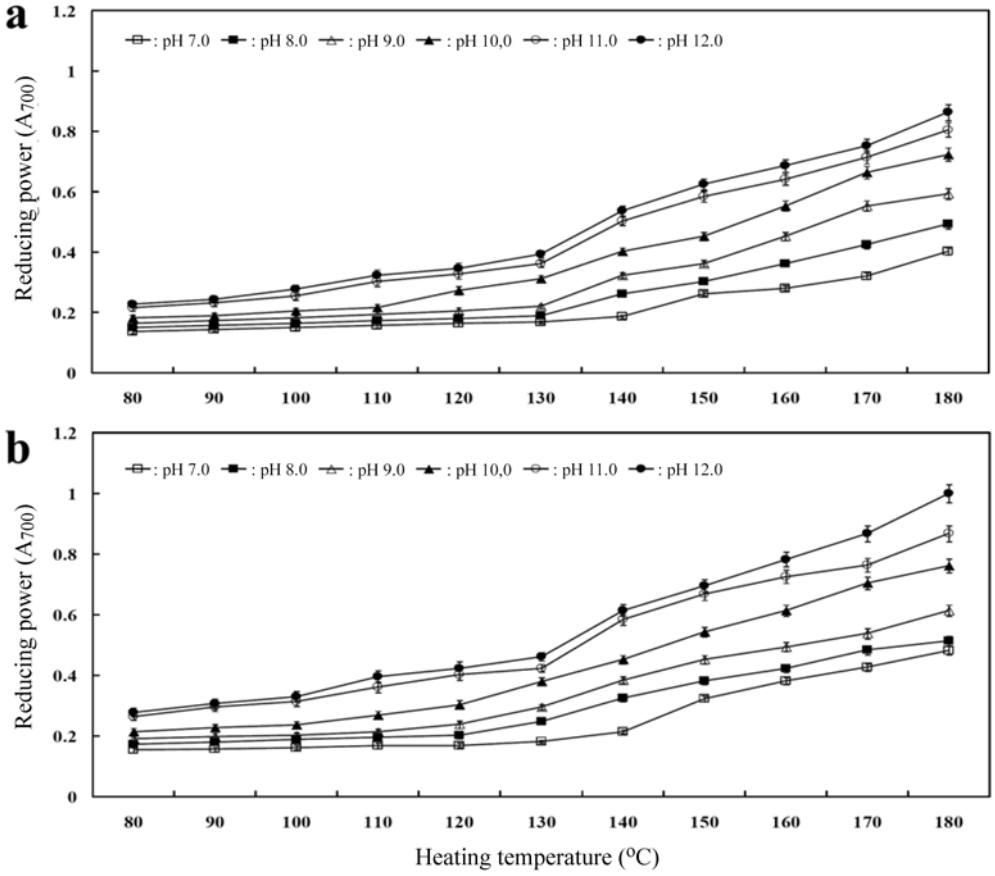

Fig. 6. Change in the reducing power of the CPs from glucose (a) and fructose (b) solutions prepared at a pH ranging from 7.0 to 12.0 for various heating temperatures (80-180°C). Vertical bars represent the standard deviation show significant ($p<0.05$) differences by Duncan's multiple range test.

Reducing power of CPs In the reducing power assay, the reductants (antioxidants) in the test samples reduce Fe^{3+} in the ferricyanide complex to its ferrous form (Fe^{2+}), which can be monitored by measuring the absorbance of Perl's Prussian blue color at 700 nm (32). Reducing power is generally associated with the presence of reductones (33), which break the free radical chain by donating a hydrogen atom (28) or react with certain peroxide precursors, thus preventing peroxide formation (34). In the present study, the reducing power of the CPs, obtained from both sugar solutions prepared at different pH level increased with increased with the increase in heating temperature and pH levels (Fig. 6) than those at higher pH levels ($p<0.05$). At all the pH levels, the reducing power of the CPs from fructose was slightly higher than that of the CPs from glucose. The results reveal that the reducing power of the CPs increased sharply after the initial heating temperature, particularly at a pH of 11.0 and 12.0 ($p<0.05$). A gradual increase in the reducing power was noted at a pH ranging from 7.0 to 9.0, but the rate of increase was less when the pH was low, than when the pH was high. The reducing power was used as an indicator of hydrogen-donating ability (17). Benjakul *et al.* (17) reported that the CPs prepared at pH 7.0 possessed lower reducing powers than those prepared at pH 10.0. The reducing power of the CPs rapidly increased after the solution was heated to 130°C. When the sugar solutions are being heated, especially under alkaline conditions, reducing compounds might be formed, and these could exhibit antioxidant activity. The antioxidant activity of the MRPs is associated with their reducing power (3). The reducing power of the CPs might be due to their hydrogen donating ability (35). Therefore, the results indicate that the increase in reducing power was occurs simultaneously with the development of browning and with the formation of intermediate products. This indicated that both brown pigments and colorless intermediates had hydrogen donating abilities, that varied with pH, sugar type, and heating temperature.

In conclusion, this study aimed to investigate the enolization reaction of the sugars glucose and fructose and the antioxidant activity of their products obtained by the in caramelization of these sugar solutions prepared at a pH ranging from 7.0 to 12.0 at various heating temperatures. To summarize, the CPs from fructose solutions that were prepared under alkaline conditions exhibited the highest degree of enolization, radical scavenging activity, and reducing power. In particular, the CPs from the fructose solutions that were prepared at pH 12.0 showed the highest antioxidant activity and could be used as promising antioxidants.

Table 1. Changes in the absorbance ratio (A_{270}/A_{420}) of the CPs from both sugars increased with increasing heating temperature and pH levels

Temperature (°C)	pH, D-Gglucose						pH, D-Fructose					
	7	8	9	10	11	12	7	8	9	10	11	12
80	1.03	1.03	1.01	1.03	1.02	1.05	1.05	1.03	1.01	1.10	1.06	1.18
90	1.00	0.95	1.00	0.98	1.03	1.19	1.03	1.03	1.02	1.12	1.37	1.73
100	1.02	1.00	1.01	1.06	1.44	1.98	1.15	0.98	1.03	1.46	1.89	2.06
110	0.98	0.99	1.03	1.52	1.80	1.85	1.07	1.03	1.15	1.51	1.59	1.64
120	1.01	0.95	1.12	1.59	1.49	1.62	1.21	1.28	1.50	1.62	1.41	1.50
130	1.10	1.07	1.47	1.46	1.45	1.67	2.19	1.38	1.57	1.77	1.53	1.70
140	1.97	1.44	1.78	1.82	1.71	1.79	3.82	1.61	1.95	1.75	1.75	1.78
150	3.86	1.75	2.05	1.79	1.74	1.81	2.67	2.01	1.55	1.72	1.71	1.86
160	3.11	2.03	1.96	1.74	1.73	1.81	2.29	1.74	1.66	1.73	1.70	1.87
170	2.58	2.02	1.93	1.76	1.71	1.72	2.15	1.80	1.83	1.76	1.65	1.65
180	2.14	1.97	1.94	1.69	1.58	1.38	2.11	1.93	1.88	1.43	1.41	1.46

Table 2. Changes in the absorbance ratio (A_{285}/A_{420}) of the CPs from both sugars increased with increasing heating temperature and pH levels

Temperature (°C)	pH, D-Glucose						pH, D-Fructose					
	7	8	9	10	11	12	7	8	9	10	11	12
80	0.91	0.90	0.87	0.90	0.88	0.92	0.92	0.90	0.88	0.95	0.98	1.18
90	0.88	0.83	0.85	0.87	0.93	1.17	0.91	0.90	0.88	1.06	1.49	2.05
100	0.89	0.88	0.87	0.98	1.56	2.34	0.97	0.85	0.92	1.65	2.32	2.56
110	0.85	0.86	0.90	1.72	1.94	2.15	1.04	0.93	1.11	1.73	1.82	1.86
120	0.88	0.81	1.05	1.79	1.57	1.73	1.17	1.23	1.58	1.75	1.49	1.62
130	0.98	0.97	1.55	1.58	1.45	1.73	2.35	1.47	1.74	2.08	1.53	1.80
140	2.02	1.48	1.92	1.88	1.79	1.83	3.98	1.70	1.78	1.81	1.74	1.80
150	3.92	1.80	2.01	1.80	1.68	1.78	2.66	1.83	1.59	1.75	1.65	1.78
160	2.97	1.90	1.89	1.69	1.63	1.69	2.17	1.75	1.76	1.67	1.59	1.76
170	2.31	1.84	1.75	1.61	1.54	1.55	1.92	1.75	1.71	1.57	1.45	1.58
180	1.88	1.76	1.80	1.50	1.39	1.28	1.94	1.72	1.77	1.27	1.30	1.37

Acknowledgments

This research was supported by the Kyung Hee University Research Fund in 2007 (KHU-20070661).

References

1. Manzocco L, Calligaris S, Mastrocola D, Nicoli MC, Lerici CR. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci. Tech. 11: 340-346 (2001)
2. Marales FJ, Jimenez-Perez S. Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. Food Chem. 72: 119-125 (2001)
3. Yen GC, Hsieh PP. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. J. Sci. Food Agr. 67: 415-420 (1995)
4. Wijewickreme AN, Kitts DD, Durance TD. Reaction condition influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. J. Agr. Food Chem. 45: 4577-4583 (1997)
5. Yoshimura Y, Iijima T, Watanabe T, Nakazawa H. Antioxidative effect of Maillard reaction products using glucose–glycine model system. J. Agr. Food Chem. 45: 4106-4109 (1997)
6. Tanaka M, Chiu WK, Nagashima Y, Taguchi T. Inhibitory effect of the Maillard reaction products towards lipid oxidation. J. Tokyo Univ. Fish. 79: 135-141 (1992)
7. Buera MP, Chirife J, Resnik SL, Wetzler G. Nonenzymatic Browning in liquid model systems of high water activity: Kinetics of color changes due to Maillard's reaction between different single sugars and glycine and comparison with caramelization browning. J. Food Sci. 52: 1063-1067 (1987)
8. Ajandouz EH, Puigserver A. Nonenzymatic browning reaction of essential amino acids: Effect of pH on caramelization and Maillard reaction kinetics. J. Agr. Food Chem. 47: 1786-1793 (1999)
9. Ajandouz EH, Tchiakpe LS, DalleOre F, Benajiba A, Puigserver A. Effect of pH on caramelization and Maillard reaction kinetics in fructose-lysine model systems. J. Food Sci. 66: 926-931 (2001)
10. Lee GC, Lee CY. Inhibitory effect of caramelization products on enzymic browning. Food Chem. 60: 231-235 (1997)
11. De Bruijn JM, Kieboom APG, Van Bekkum H. Reactions of monosaccharides in aqueous alkaline solutions. Sugar Technol. Rev. 13: 21-52 (1986)
12. Brands CMJ, Vanboekel MAJS. Reactions of monosaccharides during heating of sugar-casein system: Building of a reaction network model. J. Agr. Food Chem. 49: 4667-4675 (2001)
13. Coca M, Garcia MT, Gonzalez G, Pena M, Garcia JA. Study of coloured components formed in sugar beet processing. Food Chem. 86: 421-433 (2004)
14. O'Beirne D. Effect of pH on nonenzymatic browning during storage in apple juice concentrate prepared from bramley's seedling apples. J. Food Sci. 51: 1073-1074 (1986)
15. Myers DV, Howell JC. Characterization and specification of caramel colours: An overview. Food. Chem. Toxicol. 30: 356-363 (1992)
16. Clarke MA, Edye LA, Eggleston G. Sucrose decomposition in aqueous solution and losses in sugar manufacture and refining. Adv. Carbohyd. Chem. Bi. 52: 441-470 (1997)

17. Benjakul S, Visessanguan W, Phongkanpai V, Tanaka M. Antioxidative activity of caramelisation products and their preventive effect on lipid oxidation in fish mince. Food Chem. 90: 231-239 (2005)
18. Rhee C, Kim DH. Antioxidative activity of acetone extracts obtained from a caramelization-type browning reaction. J. Food Sci. 40: 460-462 (1975)
19. Oyaizu M. Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. Nippon Shokuhin Kogyo Gakk. 35: 771-775 (1986)
20. Yang BY, Montgomery R. Alkaline degradation of glucose: Effect of initial concentration of reactants. Carbohyd. Res. 280: 27-45 (1996)
21. Kroh LW. Caramelisation in food beverages. Food Chem. 51: 373-379 (1994)
22. Lerici CR, Barbanti D, Manzano M, Cherubin S. Early indicators of chemical changes in foods due to enzymic or non enzymic browning reactions. 1: Study on heat treated model system. Lebensm.-Wiss. Technol. 23: 289-294 (1990)
23. Homoki-Farkas P, Orsi F, Kroh LW. Methylglyoxal determination from different carbohydrates during heat processing. Food Chem. 59: 157-163 (1997)
24. Cammerver B, Wedzicha BL, Kroh LW. Nonenzymatic browning reactions of retro-aldol degradation products of carbohydrates. Eur. Food Res. Technol. 209: 261-265 (1999)
25. Buera MP, Chirife J, Resnik SL, Lozano RD. Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to caramelization of various single sugars. J. Food Sci. 52: 1059-1062 (1987)
26. Ozcelik B, Lee JH, Min DB. Effect of light, oxygen, and pH on the absorbance of 2,2-diphenyl-1-picrylhydrazyl. J. Food Sci. 68: 487-490 (2003)
27. Matthaus B. Antioxidant activity of extracts obtained from residues of different oilseeds. J. Agr. Food Chem. 50: 3444-3452 (2002)
28. Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of *Phellinus baumii* extracts. Food Chem. 82: 593-597 (2003)
29. Shih PW, Lai PL, Jen HWK. Antioxidant activities of aqueous extracts of selected plants. Food Chem. 99: 775-783 (2006)
30. Yomauchi R, Tatsumi Y, Asano M, Kato K, Ueno Y. Effect of metal salts and fructose on the autoxidation of methyl linoleate in emulsions. Agr. Biol. Chem. 52: 849-850 (1988)
31. Kirigaya N, Kato H, Fujimaki M. Stuidies on antioxidant of nonenzymatic browning reaction products. Part 1. Relation of color intensity and reductones with antioxidant activity of browning reaction products. Agr. Biol. Chem. 32: 287-290 (1968)
32. Chung YC, Chang CT, Chao WW, Lin CF, Chou ST. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. J. Agr. Food Chem. 50: 2454-2458 (2002)
33. Duh PD. Antioxidant activity of burdock (*Arctium lappa Linne*): Its scavenging effect on free radical and active oxygen. J. Am. Oil Chem. Soc. 75: 455-461 (1998)
34. Xing R, Liu S, Guo Z, Yu H, Li C, Ji X, Feng J, Li P. The antioxidant activity of glucosamine hydrochloride *in vitro*. Bioorgan. Med. Chem. 14: 1706-1709 (2006)
35. Shimada K, Fujikawa K, Yahaa K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agr. Food Chem. 40: 945-948 (1992)