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Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH

Wittayachai Lertittikul [a], Soottawat Benjakul [a,*], Munehiko Tanaka [b]

[a] Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
[b] Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Konan 4, Minato, Tokyo 108-8477, Japan

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Abstract

Maillard reaction products (MRPs) were prepared by heating the solution containing 2% porcine plasma protein (PPP) and 2% glucose adjusted to various pHs (8, 9, 10, 11 and 12) at 100 °C for different times (0, 2, 4, 6 and 8 h). The pH of all MRPs markedly decreased within 2 h of heating time. Browning and intermediate products, as monitored by absorbance at 420 nm and absorbance at 294 nm, sharply increased within 2 h ($P < 0.05$). Thereafter, slight increases were observed up to 8 h. Fluorescence intensity (Ex 347 and Em 415 nm) sharply increased within the first 2 h with a subsequent decrease when heating time increased ($P < 0.05$). Increases in browning and formation of intermediate products were concomitant with the decreases in free amino group and reducing sugar contents. Among all MRPs tested, those derived from the PPP–glucose system at pH 12 rendered the highest browning and intermediate products. However, no differences in reducing power or DPPH radical-scavenging activity of MRPs with initial pH ranges of 10–12 were noticeable. Electrophoretic study revealed that cross-linked proteins with high molecular weight were formed in the PPP–glucose model system to a greater extent at pHs 8 and 9, than at pHs 10–12. Nevertheless, heating times had no pronounced effect on protein pattern of glycated proteins.

Keywords: Maillard reaction; Browning; Antioxidant; Glycation; Radical scavenging; Porcine plasma protein

1. Introduction

The oxidation reaction directly affects food quality and is commonly associated with changes in flavour and texture. Therefore, the prevention of lipid oxidation has been of concern in the food industry. The use of synthetic antioxidants is an old practice; however, their safety could be questioned by the consumer. As an alternative, natural compounds with efficient antioxidative activity have been paid increasing attention. Products of browning reactions, both Maillard reaction and caramelisation, have been shown to possess the antioxidant activity, with free radical-scavenging activity as well as chelating properties (Benjakul, Visessanguan, Phongkanpai, & Tanaka, 2005a; Morales & Jimenez-Perez, 2001; Yoshimura, Iijima, Watanabe, & Nakasawa, 1997).

The Maillard reaction involved in the formation of brown pigments comprises the condensation between a carbonyl group of reducing sugars, aldehydes or ketones and an amine group of free amino acids (such as amino acids, peptides and proteins) or any nitrogenous compound (Jing & Kitts, 2002; Yoo, Kim, Kim, & Kang, 2004). The Maillard reaction, produced from a amino acid–sugar model system, has been known to be associated with the formation of compounds with pronounced antioxidant activity (Yoshimura et al., 1997). Antioxidative activities of Maillard reaction products (MRPs) were

* Corresponding author. Tel.: +66 74 286334; fax: +66 74 212889.
E-mail address: soottawat.b@psu.ac.th (S. Benjakul).

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affected by pH and temperature used (Mastrocola & Munari, 2000). The pH strongly influences the proportion of the amino acid in the unprotonated form and thus the initial condensation step of the Maillard reaction is augmented by higher pH. Higher pH favours the reductone formation over furfural production from the Amadori products, leading to colour development (Bates, Ames, Mac Dougall, & Taylor, 1998). Generally, brown colour developed is correlated well with the antioxidant activity (Benjakul, Lertittikul, & Bauer, 2005b; Morales & Jimenez-Perez, 2001).

Porcine blood is an abundant by-product in the slaughtering process in Thailand with an estimated amount of 30,000 metric tons per year (Benjakul, Visessanguan, & Srivilai, 2001a). Blood plasma contains a variety of bioactive compounds, including a proteinase inhibitor and plasma transglutaminase (Benjakul et al., 2001a; Benjakul, Visessanguan, & Srivilai, 2001b). The addition of porcine blood plasma could increase the breaking force and deformation of bigeye snapper surimi gel (Benjakul et al., 2001a). Apart from utilisation as surimi gel enhancer, porcine plasma protein (PPP) can be used as the potential source of proteins or peptides for Maillard reaction in a PPP–sugar model system (Benjakul et al., 2005b). MRPs from a bovine serum albumin-oxidation product of the methyl linoleate model system, prepared at pH 10, exhibited the greater antioxidative activity than that obtained at pH 7 (Alaiz, Hidalgo, & Zamora, 1999). To maximise antioxidative effect of MRPs, alkaline pH would be a promising condition, rendering MRPs with pronounced activity. So far, the information regarding the characteristics, particularly protein pattern, as well as antioxidative activity of MRPs derived from the PPP–sugar model system, is scarce. The objective of this investigation was to study the effect of pH on characteristics and antioxidative activity of MRPs from the PPP–glucose model system.

2. Materials and methods

2.1. Chemicals

2,4,6-Trinitrobenzenesulfonic acid (TNBS), L-leucine, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), sodium arsenate, and potassium ferricyanide were purchased from Sigma–Aldrich (St. Louis, MO, USA). Trichloroacetic acid, potassium sodium tartrate and ammonium molybdate were obtained from Riedel-deHaen (Seelze, Germany). Glucose and other chemicals were purchased from Merck (Damstadt, Germany).

2.2. Preparation of porcine plasma protein (PPP)

PPP was prepared according to the method of Benjakul et al., 2005b. Porcine blood was collected from a slaughter house in Hat Yai, Thailand. The blood was mixed with 3.8% trisodium citrate at a ratio of 1:9 (v/v) to prevent blood coagulation and transported on ice to the Department of Food Technology, Prince of Songkla University, Hat Yai. The blood was then centrifuged at 1000*g* for 30 min at 4 °C using a Sorvall Model RC-5B Plus centrifuge (Newtown, CT, USA) to remove red blood cells. The resultant supernatant was dialysed with 10 volumes of distilled water using seamless cellulose tubing with 8 kDa MW cut-off (Shiraimatsu Co., Tokyo, Japan) at 4 °C for three times. Dialysed plasma protein was freeze–dried and kept at 4 °C until used. The dry powder was referred to as PPP.

2.3. Preparation of Maillard reaction products (MRPs)

PPP (2 g) and glucose (2 g) were dissolved in 90 ml of different buffers (0.05 M sodium phosphate buffer, pH 8 and 9; 0.05 M sodium hydrogen carbonate buffer, pH 10, 11 and 12). The pH of solution was readjusted and the volume was brought to 100 ml using the same buffer. The solutions (10 ml) were then transferred to 25-ml screw-sealed tubes (Pyrex®, USA), tightly capped and heated in an oil bath at 100 °C. The samples were taken out after heating for 0, 2, 4, 6 and 8 h. The heated samples were cooled immediately in iced water. MRP samples obtained were kept at 4 °C until analysed. All model systems were prepared in triplicate.

2.4. Measurement of UV-absorbance and browning

The UV-absorbance and browning of MRP samples were measured according to the method of Ajandouz, Tchiakpe, Ore, Benajiba, and Puigserver (2001). Appropriate dilution (10–80-fold) was made using distilled water and the absorbance was measured at 294 and 420 nm using a UV-1601 spectrophotometer (Shimadzu, Kyoto, Japan) for UV-absorbance and browning intensity, respectively.

2.5. Measurement of fluorescence

Fluorescence of MRP samples with 10-fold dilution was determined as described by Morales and Jimenez-Perez (2001). The fluorescence intensity was measured at an excitation wavelength of 347 nm and an emission wavelength of 415 nm using a RF-1501 Fluorescence spectrophotometer (Shimadzu, Kyoto, Japan).

2.6. Determination of free amino group content

Free amino group content was determined according to the method of Benjakul and Morrissey (1997). MRP samples (5-fold dilution) (125 μl) were mixed with 2.0 ml of 0.21 M phosphate buffer, pH 8.2, and 1.0 ml of 0.01% TNBS solution was then added. The solutions were mixed thoroughly and placed in a temperature-controlled water bath (Memmert, Bavaria, Germany) at 50 °C for 30 min in the dark. The reaction was terminated by adding 2.0 ml of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The blank was prepared

in the same manner as the samples except that distilled water was used instead of 0.01% TNBS. The absorbance was measured at 420 nm. Free amino group content was expressed in terms of L-leucine.

2.7. Determination of reducing sugar content

Reducing sugar content was determined according to the method of Chaplin (1994). All reagents were prepared as described by Chaplin (1994). One ml of MRP samples (400-fold dilution) was mixed with 1.0 ml of reagent C in screw-sealed tubes. The mixtures were heated in a boiling water for 15 min and then cooled with tap water. One ml of reagent D was added and mixed well. Finally, 3 ml of deionised water was added to the mixtures. The absorbance was measured at 520 nm. The reducing sugar content was calculated from the standard curve.

2.8. Determination of reducing power

The reducing power of MRP samples was determined according to the method of Oyaizu (1986) with a slight modification. One ml of MRP sample (5-fold dilution) was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged at 750*g* using Mikro 20 centrifuge (Hettick zentrifugen, Germany) for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 μl of 0.1% $FeCl_3$. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A_{700} after blank substraction.

2.9. Determination of DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined according to the method of Yen and Hsieh (1995) with a slight modification. To 400 μl of MRP samples (20-fold dilution), 2 ml of 0.12 mM DPPH in methanol was added. The mixture was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of mixtures was measured at 517 nm using a UV-1601 spectrophotometer. The control was prepared in the same manner except that deionised water was used instead of MRP samples. The blank was conducted in the same fashion but deionised water was used instead of DPPH solution. DPPH radical-scavenging activity was calculated as follows (Singh & Rajini, 2004):

$$\text{Radical scavenging activity } (\%) = (1 - (A_{\text{sample (517 nm)}}/A_{\text{control (517 nm)}})) \times 100,$$

where $A_{\text{sample (517 nm)}}$ is the absorbance of sample after blank substraction and $A_{\text{control (517 nm)}}$ is the absorbance of the control.

2.10. SDS–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed using 4% stacking gel and 10% running gel according to the method of Laemmli (1970) with a vertical gel electrophoresis unit (Mini-Protein II; Bio-Rad Laboratories, Richmond, CA, USA). Protein (15 μg) was applied to the gel. The electrophoresis was carried out at 15 mA. After separation, protein bands were stained using Coomassie Brillant Blue R-250 (0.2%) in 25% methanol and 10% acetic acid. Destaining was performed using 40% methanol and 10% acetic acid.

2.11. Protein determination

Protein concentration was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951). Bovine serum albumin was used as the standard.

2.12. Statistical analysis

All analyses were run in triplicate. Analysis of variance (ANOVA) was performed and mean comparisons were carried out by Duncan's multiple range test (Steel & Torrie, 1980). Analysis was performed using a SPSS package (SPSS 10.0 for windows, SPSS Inc, Chicago, IL).

3. Results and discussion

3.1. Changes in pH

The pHs of all PPP–glucose MRPs with different initial pHs decreased markedly from their initial values within the first 2 h ($P < 0.05$). Thereafter, pH slightly decreased up to 8 h of heating time ($P < 0.05$) (Fig. 1). The reduction of pH occurring in the Maillard reaction was due to the formation of organic acids, such as formic and acetic acid (Ames, 1998; Brands & Van Boekel, 2002). From the result, a higher initial pH rendered a greater final pH of MRPs at every heating time used. The result was in accordance with Apriyantono and Ames (1993), Morales and Jimenez-Perez (2001) and Benjakul et al. (2005b) who found that, during the Maillard reaction, pH frequently decreased as the heating time increased.

3.2. Changes in A_{294}

A sharp increase in A_{294} of all MRP samples was observed after 2 h of heating ($P < 0.05$) (Fig. 2(a)). Nevertheless, small changes in A_{294} of all MRP samples were found with increasing heating times up to 8 h. The result suggested that intermediate products were produced to a great extent within the first 2 h. During extended heating,

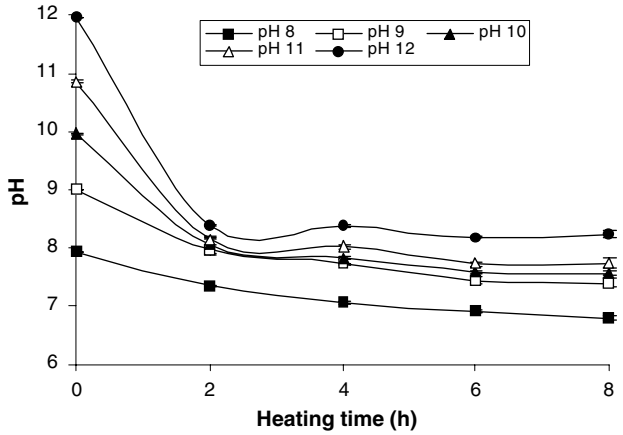

Fig. 1. Changes in pH of PPP–glucose MRPs with various initial pHs during heating for different times. Bars indicate the SD from triplicate determinations.

some intermediate products might undergo polymerisation to form the brown pigments and thus only a small amount of intermediate products was generated. The higher A_{294} observed in MRPs with higher initial pHs suggested that the intermediate products were favourably formed at alkaline pH. This result was in accordance with Moreno, Molina, Olano, and Lopez-Fandino (2003), who found that a higher pH of the glucose–lysine model system showed a higher A_{294}. Moreover, a higher pH value gave a higher A_{294} in the fructose–lysine system at 100 °C (Ajandouz et al., 2001).

3.3. Changes in fluorescence intensity

Fluorescence intensity of all MRPs with different initial pHs reached a maximum within 2 h of heating time ($P < 0.05$) (Fig. 2(b)). Subsequently, a gradual decrease was observed up to 8 h. Recently, Benjakul et al. (2005b) found a sharp increase in fluorescence intensity of PPP–sugar model systems with initial pH of 7.6–7.8 within the first hour of heating, followed by a continuous decrease up to 5 h of heating. Jing and Kitts (2004) found that the fluorescence intensity of sugar–lysine model systems heated at 121 °C and pH 9 quickly reached a maximum within 0.5 h before a subsequent decrease up to 2 h of heating time. Additionally, a similar result was also found in the ribose–casein system (Jing & Kitts, 2002). From the result, MRPs with higher initial pH showed lower fluorescence intensity at all heating times used. However, the fluorescence intensity in an epoxyaldehyde–lysine model system incubated at 25 °C for 2 h was higher at higher pHs (Hidalgo & Zamora, 1993). Generally, increase in pH of the system influenced the Maillard reaction rate (Davidek, Clety, Aubin, & Blank, 2002; Jing & Kitts, 2002; Martins, Marcelis, & Van Boekel, 2003). The Maillard reaction is associated with the development of fluorescent compounds formed prior to the generation of brown pigments (Baiser & Labuza, 1992; Morales, Romero, & Jimenez-Perez, 1996). This fluorescent compounds may be precursors of

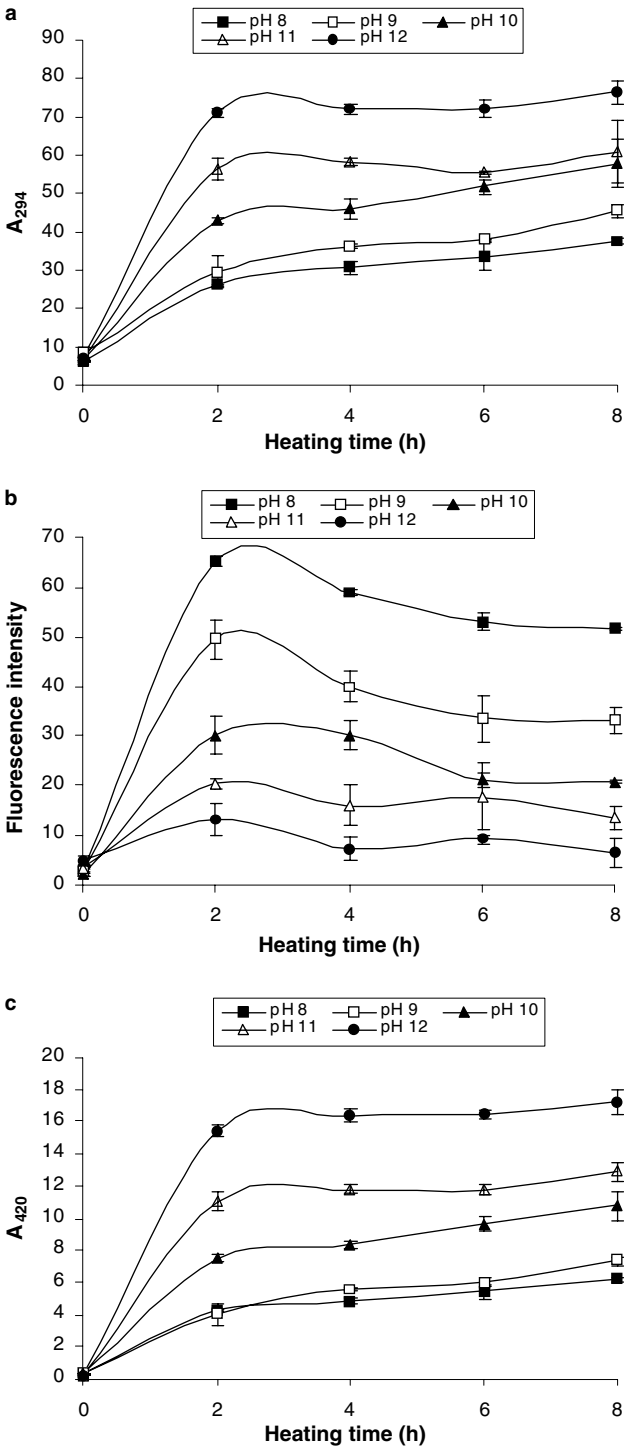

Fig. 2. Changes in A_{294} (a), fluorescence intensity (b) and browning intensity (c) of PPP–glucose MRPs with various initial pHs during heating for different times. Bars indicate the SD from triplicate determinations.

brown pigments (Labuza & Baisier, 1992; Morales & Van Boekel, 1997). The lowest fluorescence intensity in MRPs with the highest initial pH (pH 12) was probably caused by the rapid transformation of the intermediates to brown compounds. This led to less remaining fluorescent intermediate products, as shown by the lowest fluorescence

intensity. Nevertheless, the opposite result was found, when compared with results of A_{294}. Thus, those intermediate products might be different in terms of reactivity to form brown pigment. From the result, it was postulated that fluorescent compounds might undergo the polymerisation to form brown pigment much faster than those with the UV absorbing property.

3.4. Changes in browning intensity

The browning intensity, as measured by A_{420}, increased sharply within 2 h of heating ($P < 0.05$) (Fig. 2(c)). Subsequently, no differences in browning intensity of MRP samples with initial pH of 11 and 12 were found when heated for 4–8 h ($P > 0.05$). However, slight increases in browning intensities of MRP samples with initial pHs of 8, 9 and 10 were observed after 2 h of heating time ($P < 0.05$). From the result, MRP samples with an initial pH of 12 had the highest browning intensity, followed by those with initial pHs of 11, 10, 9 and 8, respectively. The result was in agreement with Ajandouz et al. (2001) who found that higher browning intensities of the fructose–lysine system heated at 100 °C were observed with increasing pH values. From the result, the browning intensities of all samples, especially with high pH, increased with the concomitant increase in A_{294} (Fig. 2(a)). Nevertheless, a low fluorescence intensity was observed, suggesting more ability of the fluorescent compound to form brown pigments. Generally, the UV-absorbing and colourless compounds formed at intermediate stages contributed to the brown pigment formation in both Maillard and caramelisation reactions (Ajandouz et al., 2001; Benjakul et al., 2005a, 2005b; Mauron, 1981).

3.5. Changes in free amino group content

The free amino group content in the PPP–glucose system, at all initial pHs, sharply decreased within the first 2 h ($P < 0.05$) (Fig. 3(a)). No differences in free amino group content were observed with increasing heating time. However, a continuous decrease in free amino group content of MRP samples with initial pHs of 8 and 9 was noted within 4 h of heating ($P < 0.05$). The result indicated that the Maillard reaction took place more effectively at high pH, as evidenced by the lower amino group content remaining. The decrease in amino group content was coincidental with increase in browning intensity (Fig. 2(c)). The reduction of free amino groups (the reactant of the Maillard reaction) was also observed in sugar-amino systems (Baxter, 1995; Bell, 1996; Sun, Hayakawa, & Izumori, 2004; Wahyuni, Ishizaki, & Tanaka, 1999). Our result was in accordance with Ajandouz et al. (2001) who reported that free amino groups in the fructose–lysine system heated at 100 °C were sharply decreased when a high initial pH was used. Therefore, PPP might serve as a source of reactive amino groups for the Maillard reaction. Since lysine has two α- and ε-amino groups, it may have higher reactivity than other amino acids. Conversely, the sulfur amino acids and peptides, such as cysteine and glutathione, are generally effective for inhibiting nonenzymatic browning (Kwak & Lim, 2004).

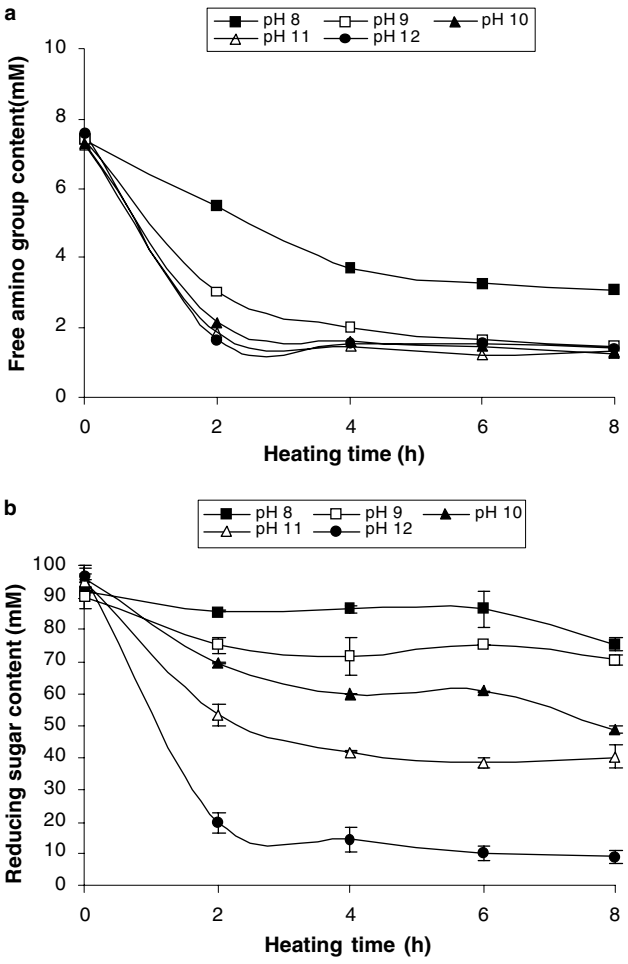

Fig. 3. Changes in free amino groups (a) and reducing sugar (b) of PPP–glucose MRPs with various initial pHs during heating for different times. Bars indicate the SD from triplicate determinations.

3.6. Changes in reducing sugar content

Sharp decreases in reducing sugar in the PPP–glucose system with initial pHs of 10, 11 and 12 were noticeable at 2 h of heating ($P < 0.05$). A gradual decrease was found when heated up to 8 h ($P < 0.05$) (Fig. 3(b)). For the MRPs with initial pHs of 8 and 9, the reducing sugar content slightly decreased as the heating time increased. The greatest decrease in reducing sugar content was observed in MRPs with an initial pH of 12. This result indicated that the reducing sugar was used as a reactant in the Maillard reaction and the reaction rate was faster in a higher pH system than lower. Reducing sugar reduction in heated fructose–lysine (Ajandouz et al., 2001) and glucose–glycine (Van Boekel & Martins, 2002; Martins & Van Boekel, 2005) model systems was influenced by initial pH values. Apart from the Maillard reaction, the caramelisation of glucose, which contributes to nonenzymatic browning

reactions, also takes place at the same time (Benjakul et al., 2005a, 2005b). At high temperature and pH, the caramelisation reaction resulted in the degradation of reducing sugar (Ajandouz et al., 2001).

The decreases in free amino group content (Fig. 3(a)) and reducing sugar content (Fig. 3(b)) of the PPP–sugar model system, especially at pH 12, were in accordance with the increases in browning intensity (Fig. 2(c)), A_{294} (Fig. 2(a)) and the lowered fluorescence intensity (Fig. 2(b)). The result revealed that a higher pH induced the condensation reaction between free amino groups of PPP and the carbonyl groups of glucose, particularly within the first 2 h of heating time.

3.7. Changes in reducing power

Reducing powers of all MRPs with different initial pHs and heating times sharply increased within the first 2 h of heating ($P < 0.05$) (Fig. 4(a)). No differences in reducing power were observed between MRPs with the initial pHs of 10, 11 and 12 when the heating time was more than 2 h. MRPs with the initial pH of 8 showed the lowest reducing power at all heating times (compared with those with the higher pHs). MRPs prepared from PPP–glucose model systems with initial pHs of 10, 11 and 12 had similar reducing powers at all heating times used. MRPs from xylose–lysine (Yen & Hsieh, 1995), glucose–glycine (Yoshimura et al., 1997), sugar–lysine (Wijewickreme, Krejpcio, & Kitts, 1999) and PPP–sugar (Benjakul et al., 2005b) model systems possessed reducing power. The result revealed that MRPs from the PPP–glucose model system, especially with high initial pH, had hydrogen-donating activity. The hydroxyl groups of MRPs play an important role in reducing activity (Yoshimura et al., 1997). Additionally, the intermediate reductone compounds of MRPs were reported to break the radical chain by donation of a hydrogen atom (Eichner, 1981). From these results, reducing powers of MRPs with various initial pHs correlated well with browning intensity (Fig. 2(c)).

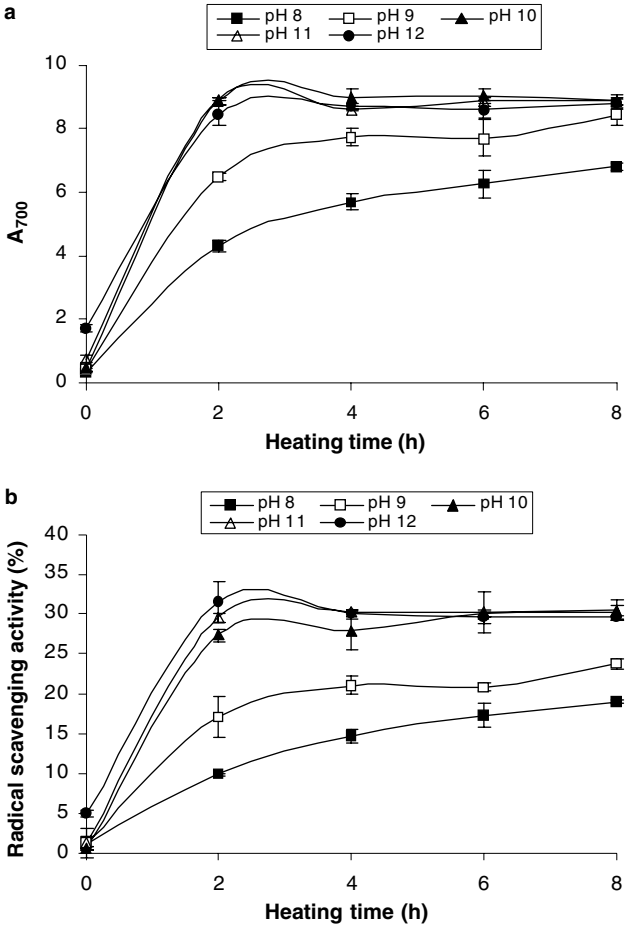

Fig. 4. Reducing power (a) and DPPH radical-scavenging activity (b) of PPP–glucose MRPs with various initial pHs during heating for different times. Bars indicate the SD from triplicate determinations.

3.8. Changes in DPPH radical-scavenging activity

The scavenging activity of PPP–glucose MRPs on DPPH radical, a molecule containing a stable free radical, is depicted in Fig. 4(b). The DPPH radical-scavenging activity indicates the hydrogen-donating abilities of antioxidants (Brand-Williams, Cuvelier, & Barset, 1995; Guerard & Sumaya-Martinez, 2003). DPPH radical-scavenging activity of MRPs with initial pHs of 10, 11 and 12 sharply increased at 2 h of heating (27.4–31.6% scavenging activity) ($P < 0.05$) and no changes in activity were found with increasing heating up to 8 h ($P > 0.05$) (Fig. 4(b)). Nevertheless, continuous increases in radical-scavenging activity were observed in MRPs with initial pHs of 8 and 9 when heating time was increased ($P < 0.05$). This result indicated that MRPs derived from the PPP–glucose model system were free radical inhibitors, which can work as the primary antioxidant. The result was in agreement with Yen and Hsieh (1995), Murakami et al. (2002), Morales and Jimenez-Perez (2001) and Benjakul et al. (2005b) who also found that MRPs had DPPH radical- scavenging activity. The higher pH of the PPP–glucose model system resulted in a greater antioxidative activity of MRPs, compared with the lower pHs used. No differences in activity were noted between MRPs with initial pHs of 10, 11 and 12. In general, the radical-scavenging activity was concomitant with the reducing power observed in MRPs. Therefore, pH was the important factor determining antioxidative activity of MRPs from the PPP–glucose model system.

3.9. Changes in protein pattern

Protein patterns of porcine plasma protein after heating in the presence of glucose at different pHs are shown in Fig. 5. For procine plasma protein, a protein with the molecular weight of 63,000–64,000 Da was the major protein and that having molecular weight of 55,000 Da was the second dominant. At pHs of 8 and 9, the proteins in the PPP–glucose model system underwent polymerisation to a great extent and a very large aggregate was formed,

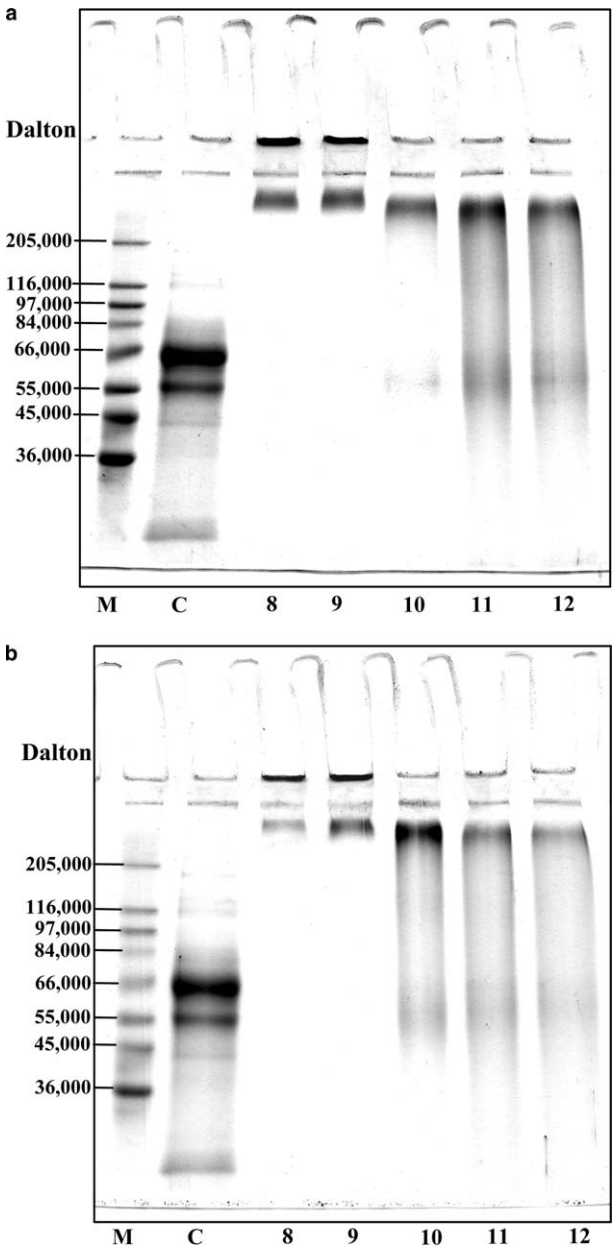

Fig. 5. Protein patterns of PPP–glucose MRPs in the model system with different initial pHs and heated for 2 h (a) and 8 h (b). M: high-molecular-weight marker; C: porcine plasma protein. Numbers denote the initial pHs of model system.

as evidenced by the dark band on the stacking gel. However, cross-links with lower molecular weight were noticeable in the PPP–glucose model system with initial pH ranges of 10–12. At all pHs used, the cross-links with molecular weight greater than 205,000 Da were generated via the Maillard reaction. Smear bands of protein with molecular weights of 55,000–65,000 Da were retained in the model system with initial pHs above 9. This result suggested that the formation of a large aggregate of both predominant proteins in porcine plasma was favoured at pH 8 and 9, in which no proteins were retained after the reaction. At the high temperature used for the reaction, proteins might be degraded to a greater extent at very alkaline pHs, compared with lower pHs. As a consequence, the cross-links with the higher molecular weight could be enhanced via the Maillard reaction at pHs 8 or 9. When β-lactoglobulin was heated in the presence of sugars, glycated protein with higher molecular weight was formed (Chevalier, Chobert, Dalgalarrondo, Choiset, & Haertlé, 2002). During glycation of β-lactoglobulin, numerous polymers with different electrophoretic patterns were formed (Bouhallab, Morgan, Henry, Mollé, & Léonil, 1999). From the result, different heating times, 2 and 8 h, had no marked effect on the protein pattern of PPP–glucose MRPs. On the other hand, pH exhibited a pronounced effect on cross-linking and pattern of PPP. Since MRPs prepared at different initial pHs showed varying antioxidative activities, the molecular weight and conformation of glycated PPP might be associated with hydrogen-donating properties or reducing power of MRPs. However, proteins undergo chemical alteration when exposed to high temperature and alkaline pH. Reaction of reducing sugar with ε-amino group also decreases the digestibility of lysine (Damodaran, 1996). Racemisation of L-amino acid residues to D-amino acid takes place at alkaline pH and high temperature (Liardon & Friedman, 1987). D-Amino acids are generally less hydrolysed by proteases (Damodaran, 1996). In addition to racemisation, the carbanion formed under alkaline pH can also undergo β-elimination reaction to yield dehydroalanine, which impairs the digestibility of protein and bioavailability of lysine (Damodaran, 1996). Therefore, minimisation of these reactions under alkaline condition is desirable to prepare MRPs with high antioxidative activity but less negative effects.

4. Conclusion

The pH of the PPP–glucose model system showed a pronounced effect on the Maillard reaction. MRPs prepared by heating PPP–glucose mixtures with higher initial pHs showed the higher antioxidant activity. Generally, antioxidative activity was coincidental with the increase in browning and absorbance at 294 nm with concomitant decrease in free amino group and reducing sugar. Initial pH of the system had a the marked influence on the cross-linking of proteins via glycation, which might be associated with antioxidative activity of MRPs. Thus, porcine plasma can be used as a potential source of amino groups for the Maillard reaction, in which MRPs with antioxidant activity are produced.

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