Characteristics and antioxidant activity of water-soluble Maillard reaction products from interactions in a whey protein isolate and sugars system

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The objective of this study was to determine antioxidant activities of water-soluble MRPs (Maillard reaction products) from the reactions between whey protein isolate (WPI) and xylose (X), glucose (G), fructose (F), lactose (L), maltose (M) and sucrose (S) at different initial pH values (3, 4, 5, 6, 7, 8 and 9). MRPs derived from the WPI-X system with increasing of pH rendered the highest browning, reducing power and DPPH radical-scavenging activity. SDS–PAGE analyses indicated formation of cross-linked proteins of large molecular mass produced from WPI-X systems. Results of FT-IR analysis indicated that the amide I, II and III bands of WPI from the WPI-X and WPI-G systems were changed by the Maillard reaction. CD spectroscopy showed that β-sheet, β-turns and random coil were increased while the α-helix was decreased after the WPI-G and WPI-X system aqueous solutions were heated. In conclusion, MRPs obtained from the WPI-X system had high antioxidant activity.

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1. Introduction

Improvement of functional properties of food proteins, using physical, chemical and/or enzymatic means, has been studied. Recently, some attempts were made to improve the functional properties of proteins through protein–saccharide graft reactions without using chemicals, which are based on Maillard reactions between the amino groups of proteins and the reducing-end carbonyl groups of saccharides (Guan, Qiu, Liu, Hua, & Ma, 2006; Kim & Lee, 2009). It has been reported that protein–saccharide grafts are useful as a new functional biopolymer having excellent emulsifying, antioxidant and antimicrobial effects for food applications (Guan et al., 2006).

Maillard reaction refers to the interaction initiated between the terminal α- or ε-amino group of lysine residues in peptides or proteins and the carbonyl moiety of reducing sugars. Maillard reaction may produce coloured or colourless reaction products, depending on the stage of the reaction, as well as other factors, such as pH, type of reactants, temperature, water activity and concentration of reactants (Billaud, Brun-Mérimée, Louarme, & Nicolas, 2004; Chang, Chen, & Tan, 2011). The antioxidative effect of Maillard reaction products was reported in 1954 (Franzke & Iwainsky, 1954). The antioxidant activities of Maillard reaction products have been extensively studied. In some studies, Maillard reaction products with antioxidative activity were identified, such as amino reductones, heterocyclic compounds, or high molecular melanoins, but most of the active antioxidants in Maillard mixtures or foods rich in Maillard reaction products are still unknown (Dittrich et al., 2009).

However, most of the information available, so far, on Maillard reactions is based on the results of model systems in which less attention was paid to proteins, such as the whey protein than to the free amino acids. Whey proteins are a group of proteins recovered from cheese manufacturing, with β-lactoglobulin and α-lactalbumin being the most abundant. Functionality of whey proteins as food ingredients has been extensively studied (Bryant & McClements, 1998). Many methods have been developed to modify whey protein, including conjugation with carbohydrates for improved interfacial properties (Dickinson & Galazka, 1991), cross-linking (via thermal aggregation or enzymatic reaction) and hydrolysis (Foegeding, Davis, Doucet, & McGuffey, 2002). To our knowledge, there is no information about the antioxidant activity of the whey protein isolate and reducing sugar heated alone under the same reactive conditions as their MRPs.

The goal of this study was to improve the antioxidant activity of whey proteins by glycation under wet reaction conditions. The influences of pH, type of sugar and the concentration of MRPs on antioxidant activity were studied. The degraded carbohydrate...
structures bound to the exposed sites of the reacting protein and the change of whey protein isolate molecules and the space were also studied. Another aim was to supply basic theory for the glycated whey protein isolate used as an antioxidant in formulated foods (as a functional ingredient) due to its radical-scavenging activity and capacity to delay oxidative deterioration and find a new use for whey protein isolate.

2. Materials and methods

2.1. Chemicals

Whey protein isolate (WPI) was purchased from Fonterra Ltd. (New Zealand) with a protein content of 92.4%. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Coomassie Brilliant Blue R-250, D-(+)-xylose, sodium dodecyl sulphate (SDS) and Tris were purchased from Sigma Chemical Co. (St. Louis, MO, USA). D-(+)-glucose (analytical grade), D-(+)-fructose (analytical grade), D-(+)-lactose (analytical grade), D-(+)-maltose (analytical grade) and D-(+)-sucrose (analytical grade) were purchased from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). The other solvents/chemicals used were of analytical grade and obtained from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China).

2.2. Preparation of Maillard reaction products

Whey protein isolate and sugar (equivalent to 2:1 m/m ratio of protein to sugar) was dissolved in distilled water at a total concentration of 60 mg/ml. Seven systems were prepared, including whey protein isolate (WPI), whey protein isolate-xylose (WPI-X), whey protein isolate-glucose (WPI-G), whey protein isolate-fructose (WPI-F), whey protein isolate-lactose (WPI-L), whey protein isolate-maltose (WPI-M) and whey protein isolate-sucrose (WPI-S). Six sugars (X, G, F, L, M and S) were dissolved in distilled water at a total concentration of 20 mg/ml. Every system was adjusted to a different pH (3, 4, 5, 6, 7, 8 and 9). The solutions were kept in a temperature-controlled water bath at 50 °C for 7 d.

2.3. Measurement of the browning

The absorbance of the heated solutions was measured, using a spectrophotometer (UNICO UV-2100, Shanghai, China) at 420 nm, as marker at the final stages of the reactions (Kim & Lee, 2008). Samples were diluted to 10 mg/ml with distilled water to obtain an absorbance value at 420 nm.

2.4. Determination of reducing power

The reducing power of MRPs samples was determined according to the method of Chawla, Chander, and Sharma (2009) with some modifications. 0.5 ml of MRPs sample was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide (K3Fe(CN)6). The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by addition of 2.5 ml of 10% trichloroacetic acid after cooling to room temperature. The mixtures were then centrifuged at 750g, using a centrifuge (TGL-16C, Anting, Shanghai, China) for 10 min at 25 °C. The supernatant obtained (2.5 ml) was treated with 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl3. The absorbance of the reaction mixture was measured at 700 nm with a UNICO UV-2100 spectrophotometer. Results were the averages of three measurements and expressed as absorbance units (AU).

2.5. Determination of DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined according to the method of Gu et al. (2009) with some modifications. An aliquot of MRPs sample (1.0 ml) was added to 4.0 ml of 0.12 mM DPPH in ethanol (95%). The solution was then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The mixture was centrifuged for 5 min at 750g if there was sediment. The absorbance of supernatant was measured at 517 nm, using a UNICO UV-2100 spectrophotometer. Results were the averages of three measurements and expressed as radical-scavenging activity (%). The percentage of DPPH radical-scavenging activity was calculated as follows:

\[ \text{Radical – scavenging activity} = \left(1 - \frac{A_{517\text{nm sample}} - A_{517\text{nm control}}}{A_{517\text{nm blank}}} \right) \times 100\% \]

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

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE was performed according to the method of Chicón, Belloque, Alonso, and López-Fandiño (2008, 2009) with slight modification, using 5% (pH 6.8) stacking gel and 12% (pH 8.8) separating gel. Samples (WPI, WPI-X, WPI-G, WPI-F, WPI-L, WPI-M, WPI-S, native pH) were mixed with sample buffer containing 2% SDS and 5% β-ME (ratio 1:8, m/v). The mixtures were then heated at 90 °C for 5 min before loading. The samples were run at 120 V and 80 mA in the Mini-PROTEAN II Electrophoresis Cell for 1.5 h. Subsequently, the gel was stained with 0.1% Coomassie Brilliant Blue R-250 in 40% methanol and 10% acetic acid mixed solution and destained in a solution containing 40% methanol and 10% acetic acid. The gel was then photographed with a CANON IXUS 430 digital camera. The protein fractions were identified, using Sigma’s Pre-stained Protein Maker.

2.7. FT-IR measurements

FT-IR spectra were determined according to the method of Śmiechowski and Stangret (2008) with some modifications. FT-IR spectra were recorded in ATR mode on a Nicolet 8700 FT-IR spectrometer, using the Turbo mode of the EverGlo infrared source. 128 scans were made with a selected resolution of 8 cm⁻¹. A single-reflection Specac Golden Gate ATR system was used, equipped with heated tungsten carbide disc, fitted with a 45 horizontal diamond crystal. The measurement chamber, isolated with KBr windows from the optical compartment of the spectrometer, was purged with dry nitrogen to ensure low water vapour and carbon dioxide residues in the spectra. The purge gas was also directed inside the ATR accessory. The temperature of the sample was kept at 28.0 ± 1.0 °C with the aid of an external Specac West 6100+ controller.

2.8. Circular dichroism (CD) spectroscopy measurements

The secondary structures of modified proteins were determined at 25 °C, using a circular dichroism spectropolarimeter (Jasco J-600, Jasco Corporation Japan) with a spectral resolution of 0.5 nm. The spectrum (190–250 nm) was recorded using a 10 nm path length quartz cell at a scan speed of 100 nm/min and sensitivity of 20 mdeg. The samples (WPI-X, WPI-G, WPI-S and WPI, native pH) were diluted to 0.2 mg/ml. CD spectra were corrected for solvent contributions and were expressed in terms of specific ellipticities.
versus wavelength. Estimation of secondary structure composition was performed using the Jasco SSE-338 Protein Secondary Structure Estimation Program (Japan Spectroscopic Co. Ltd., Tokyo, Japan), which was based on CD spectra of reference proteins of known secondary structures (Wada, Fujita, & Kitabatake, 2006).

2.9. Statistical analysis

Collected data were expressed as means ± standard deviation (SD). Analysis of variance (ANOVA) was performed and means comparisons were carried out by Student–Newman–Keuls’ tests. A value of $p < 0.05$ was considered significant. Data were analysed by using a statistical software package (SPSS for Windows, 11.5, 2002, SPSS Inc., USA).

3. Results and discussion

3.1. Changes in browning intensity

Brown colour development (A420 nm) is the easiest measurable consequence of the Maillard reaction because it offers a visual estimate. Its intensity is often used as an indicator of the extent to which the Maillard reaction takes place in foods and it symbolises an advanced stage of the Maillard reaction (Morales & Jiménez-Pérez, 2001). As can be seen from Table 1, a sharp increase in reducing power of the WPI-X system was observed with increasing pH values ($p < 0.05$). Moreover, the change of the colour intensity was higher than that of WPI ($p < 0.05$). A little higher browning intensity of the WPI-G systems was observed with increasing pH values ($p < 0.05$). Similar results were obtained by Lertittikul, Benjakul, and Tanaka (2007), although the colour intensity was higher. This difference could be related to the different initial pH used. The initial pH values of the reaction system were considered to affect the Maillard reaction (MR) significantly. In alkaline condition, a Schiff-base formed easily and promoted the MR further. Consequently, the brown components of the MR system were produced quickly. During the development of brown colour caused by the MR, caramelisation can occur simultaneously. Caramelisation reactions contribute to overall non-enzymatic browning, especially in the alkaline pH ranges (Ajandouz, Desseaux, Tazi, & Puigserver, 2008). Slight increases in browning intensities of WPI-M, WPI-F and WPI-L systems were observed with increasing pH values but these were less significant than the colour change of WPI (Table 1). Thus, the differences among different studies were possibly due to the diversity of sugars, as well as conditions used to prepare MRPs.

3.2. Changes in reducing power

Fig. 1(a) shows that the changes of reducing power of the sugars alone with different initial pH values (3, 4, 5, 6, 7, 8 and 9) after heating. The absorbance at A700 nm of all the sugar systems was under 0.1. This indicated that the sugar systems alone after heating had almost no reducing power. Except for WPI, reducing powers of all MRPs were increased with the increasing of pHs (Fig. 1(b)). When comparing the sugar systems, MRPs systems exhibited significantly higher ($p < 0.05$) reducing power activity than did corresponding sugars alone. The reducing power was increased among MRPs with the initial pHs of 5, 6, 7, 8 and 9 after heating. MRPs with the initial pH of 3 and 4 showed the lowest reducing power (compared with those with the higher initial pHs). The reducing power of the WPI-X system was sharply increased with the initial pH of 5, 6, 7, 8 and 9. No differences in reducing power were observed among MRPs with the initial pH of 8 and 9. However, this increase in reducing power was not significant when the WPI, WPI-M, WPI-S and WPI-L complexes increased. This result revealed that MRPs from the WPI-X system, especially with high initial pHs, had good hydrogen-donating activity. The hydroxyl groups of MRPs play an important role in reducing activity. 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 (Table 1).

3.3. Changes in DPPH radical-scavenging activity

The scavenging activity of WPI and sugar MRPs on the DPPH radical, is depicted in Fig. 2. The DPPH radical-scavenging activity indicates the hydrogen-donating abilities of antioxidants (Brand-Williams, Cuvelier, & Berstein, 1995). Fig. 2(a) shows the changes of DPPH radical-scavenging activity of the sugars alone with different initial pH values (3, 4, 5, 6, 7, 8 and 9) after heating. Fig. 2(b) shows the DPPH radical-scavenging activity of WPI and MRPs from WPI and sugar systems with different initial pH values. The DPPH radical-scavenging activity of MRPs systems was higher than those of the sugar systems after heating. Fig. 2(b) shows that the DPPH radical-scavenging activity of WPI-X with initial pH of 5–9 sharply increased after heating ($p < 0.05$) and the higher pH of the WPI-X system resulted in a greater antioxidative activity of MRPs, compared with the lower pHs used. Yen and Hsieh (1995) also reported the DPPH radical-scavenging activity of xylose–lysine MRPs. A slightly greater activity was found with MRPs derived from WPI-G. Therefore, MRPs possess hydrogen-donating ability, suggesting potency to react with free radicals. No significant changes in WPI, WPI-S, WPI-M were found with increasing pHs ($p > 0.05$) (Fig. 2(b)). This result was in agreement with Morales and Jiménez-Pérez (2001), who also found that MRPs had DPPH radical-scavenging activity. Therefore, initial pH values and the type of sugar were the important factors determining antioxidative activity of MRPs. Moreover, radical-scavenging activity correlated well with browning intensity at 420 nm and reducing power. The highest reducing power and radical-scavenging activity of MRPs from

<table>
<thead>
<tr>
<th>pH</th>
<th>Samples</th>
<th>WPI</th>
<th>WPI-L</th>
<th>WPI-S</th>
<th>WPI-X</th>
<th>WPI-G</th>
<th>WPI-F</th>
<th>WPI-M</th>
</tr>
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<tbody>
<tr>
<td>3</td>
<td>–</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.59 ± 0.02a</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td></td>
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<tr>
<td>4</td>
<td>–</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.64 ± 0.02a</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
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<tr>
<td>5</td>
<td>–</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.58 ± 0.06g</td>
<td>0.04 ± 0.00a</td>
<td>0.05 ± 0.00c</td>
<td>0.03 ± 0.00a</td>
<td></td>
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<tr>
<td>6</td>
<td>–</td>
<td>0.07 ± 0.23b</td>
<td>0.04 ± 0.00d</td>
<td>0.84 ± 0.08a</td>
<td>0.04 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td>0.03 ± 0.00a</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.03 ± 0.00a</td>
<td>0.05 ± 0.01c</td>
<td>0.04 ± 0.00d</td>
<td>0.90 ± 0.02d</td>
<td>0.05 ± 0.00e</td>
<td>0.04 ± 0.00a</td>
<td>0.03 ± 0.00c</td>
<td></td>
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<tr>
<td>8</td>
<td>–</td>
<td>0.05 ± 0.00b</td>
<td>0.04 ± 0.00d</td>
<td>0.95 ± 0.01a</td>
<td>0.06 ± 0.00c</td>
<td>0.04 ± 0.00a</td>
<td>0.04 ± 0.00a</td>
<td></td>
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<tr>
<td>9</td>
<td>–</td>
<td>0.06 ± 0.00d</td>
<td>0.04 ± 0.00d</td>
<td>0.94 ± 0.01a</td>
<td>0.06 ± 0.00c</td>
<td>0.04 ± 0.00a</td>
<td>0.04 ± 0.00a</td>
<td></td>
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</table>

*Different superscripts denote significant difference ($p < 0.05$).

SD: standard deviation from triplicate determinations.
the WPI-X system could be used (as an antioxidant) to prevent lipid oxidation in food products.

3.4. Gel electrophoresis (SDS–PAGE)

To further confirm whether the sugars were conjugated with WPI or not, patterns of WPI and sugars systems were investigated after MR. As shown in Fig. 3, under reducing conditions, the characteristic bands of initial WPI are displayed in (lane 2). Due to migrating below the range of the separating gel, the band of α-lactalbumin and β-lactoglobulin at the top is well visualised in Fig. 3. After MR of the WPI and sugar solutions, the feature bands of WPI were significantly diminished and a new dense band appeared on the top of the separating gel (lanes 7). The detected larger molecular mass distribution (more than 97.4 kDa) signified that a new composition was formed in the WPI-X system and the larger molecular mass was also pro-

![Fig. 1. Reducing power of sugars alone (a) and WPI and sugar MRPs (b) with various initial pH values, after heating. Bars indicate the SD from triplicate determinations.](image)

![Fig. 2. DPPH radical-scavenging activity of sugars alone (a) and WPI and sugar MRPs (b) with various initial pH values, after heating. Bars indicate the SD from triplicate determinations.](image)
duced at molecular weights of about 43 and 66.2 kDa. By contrast, the band of α-lactalbumin (under 20.1 kDa) was obviously reduced. This might be due to the conjugation of xylose and α-lactalbumin after the MR. As we can see (in lane 8), the large molecular mass of the WPI-F system was increased at molecular weights of about 43 and 66.2 kDa compared to WPI which indicated that the band of α-lactalbumin (under 20.1 kDa) was reduced. Possibly the fructose was also conjugated to the α-lactalbumin. This result was in accordance with that of Diftis and Kiosseoglou (2006), who reported that the Maillard conjugation of protein and polysaccharide resulted in the appearance of high molecular weight constituents in the protein patterns. The shift of the WPI profile caused by the glucose molecules is presented (in lane 3); the feature bands of WPI at 43 kDa and under 20.1 kDa disappeared after the MR. WPI-G systems may produce other materials that cannot be seen in the SDS–PAGE. These materials and the high molecular weight may be related to its reducing power and DPPH radical-scavenging activity (Figs. 1 and 2). However, almost no change was observed in the tests (lanes 4, 5 and 6). It seemed that the levels of Maillard reaction in WPI-S, WPI-L and WPI-M were low. This may be the reason why WPI-L, WPI-M and WPI-S systems had low reducing power and DPPH radical-scavenging activity. The SDS–PAGE also indicated that the intensity of Maillard reaction depended on the type of sugar used to modify the WPI.

3.5. FT-IR

The spectroscopic analysis of polymeric molecules, including proteins, is complex, due to the molecular vibrations arising from numerous atoms. FT-IR spectroscopy is a particularly useful technique for the study of protein–carbohydrate systems, as there are several readily identifiable regions of the mid-infrared spectrum where the chemical fingerprints of carbohydrates and proteins do not overlap significantly (Farhat, Orset, Moreau, & Blanshard, 1998; Gu et al., 2010). The most distinctive spectral features for proteins are the strong amide I and II bands centered approximately between 1650 and 1540 cm⁻¹, respectively. For carbohydrates, a series of overlapping peaks located in the region of 1180–953 cm⁻¹ results from vibration modes such as the stretching of C–C and C–O and the bending mode of C–H bonds. These are often referred to as the “saccharide” bands and are the most intense bands in the mid-infrared spectrum (Iconomidou et al., 2000; Lin, Chen, & Liang, 1999). These absorptions are weak in the spectra of most proteins (Caillard, Remondetto, & Subirade, 2009).

As can be seen in Fig. 4, the absorptions in the region of 1180–953 cm⁻¹ were stronger in WPI-X and WPI-G than in WPI and WPI-S and weaker than X and G, indicating that there seemed to be a saccharide attached to the WPI. It might be expected that the chemical changes accompanying the Maillard reaction in WPI would lead to several changes in the mid-IR spectrum as a result of the consumption of some functional groups and the appearance of others. Functional groups, including NH₂, especially from lysine, may be lost, while the amount of those associated with MRPs, such as the Amadori compound (C=O), Schiff base (C=N) and pyrazines (C–N) may be increased by the Maillard reaction (Śmiechowski & Stangret, 2008; Srivastava et al., 2011). Fig. 4 shows the IR spectra of WPI, WPI-X and WPI-G. Regions of 1650 and 1540 cm⁻¹, referred to as C=O and C–N stretching from amide I and II, were modified by the MR, because the intensity of WPI-X and WPI-G decreased compared to WPI. In addition, changes in the 1050–950 cm⁻¹ regions could correspond to side-chain vibrations from the whey protein isolate which, even though they cannot be classified as a functional specific group, also indicate alteration of protein structure. As can be seen in Fig. 4, this region was increased in WPI-X and WPI-G. However, there seemed to be no significant difference in WPI-S compared to WPI. This may be related to the low reducing power and DPPH radical-scavenging activity of WPI-S.

Finally, in proteins, there is an amide III band at 1300–1200 cm⁻¹. This band is known to be very complex and mainly arises from C–N stretching and N–H deformation. The entire spectral features of the amide III band for the WPI-X and WPI-G (Fig. 4) showed a decrease in intensity compared to WPI and WPI-S.
3.6. Secondary structure analysis by circular dichroism (CD) spectroscopy

CD spectroscopy is an optical technique that allows the detection and quantification of the chirality of molecular structures and provides information about the secondary and tertiary structures of proteins. Optical activity of $\alpha$-helix in the far-UV region permits the use of CD spectroscopy to investigate conformational changes in proteins and the CD band positions for various structures, the $\alpha$-helix; the interchain hydrogen bonded $\beta$-structure and a fully extended parallel or antiparallel arrangement of peptide chains have been reported (Iconomidou, Chryssikos, Gionis, Willis, & Hamodrakas, 2001). CD is often used to complement the more detailed structural information available from other techniques. CD measurements are fast and simple and have been used frequently to calculate the relative proportions of secondary structures.

CD spectra are shown in Table 2 which presents the spectra of initial WPI compared to WPI-G, WPI-X and WPI-S systems. The conjugates showed a significant loss of secondary structure, which indicated that the antioxidant activity of MRPs was also dependent on the type of sugar used depending on the concentration. This indicated that adding xylose and glucose could lead to the formation of hydrogen bonds between WPI and xylose and glucose molecules, which possibly weakens the intermolecular interaction (i.e. the hydrogen bonds between hydrogen atoms of amide and oxygen atoms of carbonyl) and results in a reduction of $\alpha$-helix (Srivastava et al., 2011; Sun, Yu, Zeng, Yang, & Jia, 2011). However, secondary structure change of the WPI-S system was not significant compared to the initial WPI. For example, $\alpha$-helix, $\beta$-sheet, $\beta$-turns and random coil contents are listed in Table 2. Obviously, $\beta$-sheet $\beta$-turns and random coil were increased while the $\alpha$-helix was decreased after the WPI-G and WPI-X system aqueous solutions were heat-treated. An explanation could be that adding xylose and glucose could lead to the formation of hydrogen bonds between WPI and xylose and glucose molecules, which possibly weakens the intermolecular interaction (i.e. the hydrogen bonds between hydrogen atoms of amide and oxygen atoms of carbonyl) and results in a reduction of $\alpha$-helix (Srivastava et al., 2011; Sun, Yu, Zeng, Yang, & Jia, 2011).

4. Conclusions

The initial pH values of the WPI and sugars system showed a pronounced effect on the Maillard reaction. MRPs prepared by heating (except WPI-S system), mixtures with higher initial pH showed higher antioxidant activity. The antioxidant activity of the WPI-X system was the highest of all the MRPs systems with increase of the concentration. This indicated that the antioxidant activity of MRPs was also dependent on the type of sugar used for the modification. Initial pH of the system had a marked influence on the cross-linking of proteins via glycation, which might be associated with the antioxidative activity of MRPs. The SDS-PAGE showed that a large molecular mass was produced after the Maillard reaction. The spectroscopic analysis of FT-IR indicated that amide I, II and III bands of WPI were modified by xylose and glucose. CD spectroscopy result showed that $\beta$-sheet $\beta$-turns and random coil were increased while the $\alpha$-helix was decreased after the WPI-G and WPI-X system aqueous solutions were heat-treated. For application of antioxidant activity of protein modified with functional ingredients in food, more research is needed, especially regarding the antioxidative effect of peptides derived from the proteolytic hydrolysis of glycated protein and the antioxidative action of protein hydrolyzates. This is particularly important for human applications. Thus, WPI can be used as a potential source of amino groups for production of MRPs with antioxidant activities.

References


