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Study of Maillard reaction products derived from aqueous model systems with different peptide chain lengths

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ABSTRACT

Maillard reaction products (MRPs) were prepared from aqueous model mixtures containing 0.2 M glucose and 0.2 M each of glycine, diglycine, and triglycine, heated at 100 °C, for different times (10, 60, 120, 180 and 240 min), with an initial pH control set to 7.8. The browning and intermediate products of MRPs derived from the Glu-Di model system were the most prominent. The pH of all MRP samples decreased considerably as the heating time increased ($P < 0.05$). The loss of glucose in MRPs derived from the Glu-Di model system was the highest, while the degree of sugar enolisation in MRPs derived from the Glu-Tri model system was the highest. The amino acid group content in MRPs derived from the Glu-Di model system was decreased noticeably. All MRP samples showed different absorptions in the UV–vis spectra, although they possessed similar shapes. The molecular weights of all MRP samples showed rising intensities as a function of the heating time, whereas the major peaks of each MRP sample were eluted at different retention times according to peptide chain lengths.

1. Introduction

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. The Maillard reaction is a complicated reaction that produces a large number of the so-called Maillard reaction products (MRPs), such as aroma compounds, ultra-violet absorbing intermediates, and dark-brown polymeric compounds named melanoidins (Wijewickreme, Kitts, & Durance, 1997). The Maillard reaction is influenced by many factors, including reactant concentration, temperature, time, initial pH and water activity (Naranjo, Malec, & Vigo, 1998). In particular, for the Maillard reaction experiment, a glucose/glycine model system is usually selected (Chawla, Chander, & Sharma, 2007; Martins & Van Boekel, 2005a, 2005b).

Oligopeptides have been extensively isolated and identified in both natural and artificial protein hydrolysates of foods, such as seafood, coffee beans, soy, and wheat gluten (Aaslyng et al., 1998; Ludwig, Lipke, Raczek, & Jäger, 2000). They have been recognised as precursors of the Maillard reaction, which lead to the colour of processed foods (Aaslyng et al., 1998). Moreover, these oligopeptides play an important role in preservation of the rheological properties of food (Taborda et al., 2008). Oligopeptides, as a final product of food hydrolysis (mostly milk, cheese and wine), are known to act as very good antioxidants, anticoagulants and blood pressure reducers (Hartman & Meisel, 2007). Furthermore, glycine, being the simplest, with no side chains to complicate the bonding of the proton, yet one of the most important amino acids, has long been a subject of intense theoretical and experimental investigation. Amino acids are the basic building blocks of proteins, which are formed through successive amide linkages (peptide bond) of several amino acids. Diglycine is the simplest peptide bond with all the typical characteristics of the complexing sites of higher peptides, while triglycine represents higher oligomers. Studies on simple oligoglycine like these may reveal important information regarding the peptide chain length effect in the Maillard reaction.

Although a wide range of peptides has been reported (in considerable quantity) in many food systems, such as aged sake, meat and hydrolysed vegetable protein, only a few studies have investigated the Maillard reaction products formed from oligopeptides (Oh, Hartman, & Ho, 1992; Oh, Shu, & Ho, 1991; Rizzi, 1989). Thus, employment of an oligoglycine peptide as the simplest model would be suitable for profiles on MRPs to simulate the peptide chain length effects in the Maillard reaction. This could more useful in understanding the Maillard reaction and it might be extended to the reaction of peptides with reactive carbonyl compounds in general. Therefore, the objective of this study was to evaluate the profiles of MRPs derived from aqueous model systems with different peptide chain lengths as a function of the heating time.

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2. Materials and methods

2.1. Materials

D-Glucose, glycine, diglycine, triglycine, blue dextran, bovine serum albumin, albumin, β-lactoglobulin, myoglobin, ribonuclease A and cytochrome C were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, and HPLC-grade water were purchased from J.T. Baker (Phillipsburg, NJ, USA). *o*-Phthalaldehyde was purchased from Agilent Technologies (Wilmington, DE, USA). All chemicals used were of analytical grade and were obtained from Merck (Darmstadt, Germany), unless mentioned otherwise.

2.2. Preparation of Maillard reaction products (MRPs)

Equimolar (0.2 M) amounts of glycine, diglycine and triglycine were dissolved, individually, with 0.2 M of glucose in about 90 ml of deionised water. The pH of the solution was adjusted to 7.8 with 6 N NaOH. The pH value was used to minimise peptide bond breaks. Especially, diglycine needs an optimum of pH 7.5–8.9 to maintain the operation (Budavari, O'Neil, Smith, Heckelman, & Kinneary, 1996). The solution was then brought to a final volume of 100 ml with deionised water adjusted to 7.8 with 6 N NaOH. The solutions (10 ml) were then transferred to 25 ml screw-sealed tubes (Pyrex®, USA), tightly capped and heated in a thermostatic oil bath at 100 °C. The samples were taken out after heating for 10, 60, 120, 180, and 240 min. After the heating period, the tubes were immediately cooled in ice-water. Some of the MRP samples were used directly for final pH measurements, while the remainder were kept at −4 °C until further use since storage did not alter the sugar and glycine oligopeptide contents up to 2 days. All model systems were prepared in triplicate.

2.3. Measurement of pH

The pH was measured using a Corning 440 pH metre (Corning Life Sciences, New York, USA) calibrated with buffer solutions of pH 4.0 and 10.0, respectively.

2.4. Absorbance measurements of MRPs

The UV-absorbance and browning of MRP samples were measured according to the method of Ajandouz, Tchiakpe, Ore, Benajiba, and Puigserver (2001). The absorbance of MRPs was measured using a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan) at 294 nm, as an indication of the formation of intermediate products of nonenzymatic browning, and at 420 nm, as an index of the brown polymers formed in more advanced stages.

2.5. Determination of sugars in MRPs

The reducing sugars in MRPs samples were determined using an HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, USA). An Agilent quaternary pump, connected to a refractive index detector (Hewlett Packard, Model: G1362A, Wilmington, DE, USA), was used with a Zorbax carbohydrate column (4.6 × 250 mm I.D., 5 μm particle size, Agilent Technologies, Wilmington, DE, USA). The mobile phase, consisting 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 of sample were injected into the HPLC system. The data analysis was performed using Chemstation software (Rev. A. 10.01, Hewlett Packard).

2.6. Determination of glycine, diglycine, and triglycine in MRPs

The glycine, diglycine, and triglycine in MRPs samples were analysed using an HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, USA) with a variable wavelength detector, VWD HP 1100, operating at 338 nm (excitation = 340 nm). Separation was carried out with a Zorbax Eclipse AAA Rapid Resolution column (150 × 4.6 mm I.D., 5 μm particle size, Agilent Technologies, USA). A linear gradient profile of mobile phase, comprising 40 mM Na_2H-PO_4, pH 7.8 (solvent A) and ACN/MeOH/water 45:45:10 (v/v) (solvent B), 0% B (0–1.9 min), 0–57% (1.9–18.1 min), 57–100% (18.1–18.8 min), 100% (18.8–22.3 min), 100–0% (22.3–23.2 min) and 0% (23.2–26 min) was applied at a flow rate of 2.0 ml/min. The column was equilibrated for 5 min under initial conditions prior to injection of the next samples. The column temperature was 40 °C. In order to determine amino acids from MRPs, precolumn derivatisation with *o*-phthalaldehyde (OPA) was used and 0.5 μl of prepared sample were injected into the HPLC system. The data analysis was performed using Chemstation software (Hewlett Packard).

2.7. Wavelength spectra of MRPs

Wavelength spectra of MRPs were recorded by a UV–vis spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan), with the wavelength ranging from 200 to 700 nm.

2.8. MW measurement of MRPs

The MW distribution profiles of the MRPs samples were estimated by gel permeation chromatography (GPC) using a TSK-guard column PWH (75 × 7.5 mm I.D) and a TSK-G3000PW gel filtration column (600 × 7.5 mm I.D., 10 μm particle size, TOSOH Corporation, JAPAN) with a useful fractionation range between 1000 Da and 4000 kDa. The liquid chromatographic system consisted of an Agilent quaternary pump, an Agilent G1313A variable volume autosampler and an Agilent variable wavelength detector (Hewlett Packard, Wilmington, DE, USA). The mobile phase, consisting of acetonitrile/water (75:25, v/v), was delivered at a flow rate of 1.0 ml/min. The column temperature was 30 °C. Fifty microlitres of MRPs (sterilised through 0.22 μm filtres) were injected onto the column, and elution was carried out isocratically in sodium phosphate buffer at pH 7.2 with 50 mM NaCl at a flow rate of 1 ml/min. Seven standards were used as MW calibration standards (log MW = −0.1654 Rt + 6.5726 with Rt = retention time, expressed in minutes): blue dextran (2000 kDa), bovine serum albumin (66 kDa), albumin (44.287 kDa), β-lactoglobulin (36 kDa), myoglobin (17 kDa), ribonuclease A (13.7 kDa), and cytochrome C (12.380 kDa). Chromatograms were recorded by UV-detection at 220 nm, of peptide bond rearrangements. The data analysis was performed using Chemstation software (Hewlett Packard).

2.9. Statistical analysis

All experimental data were subjected to 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).

3. Results and discussion

3.1. Changes in pH

The changes in pH of MRPs derived from aqueous glucose/glycine, diglycine and triglycine model systems, as a function of the

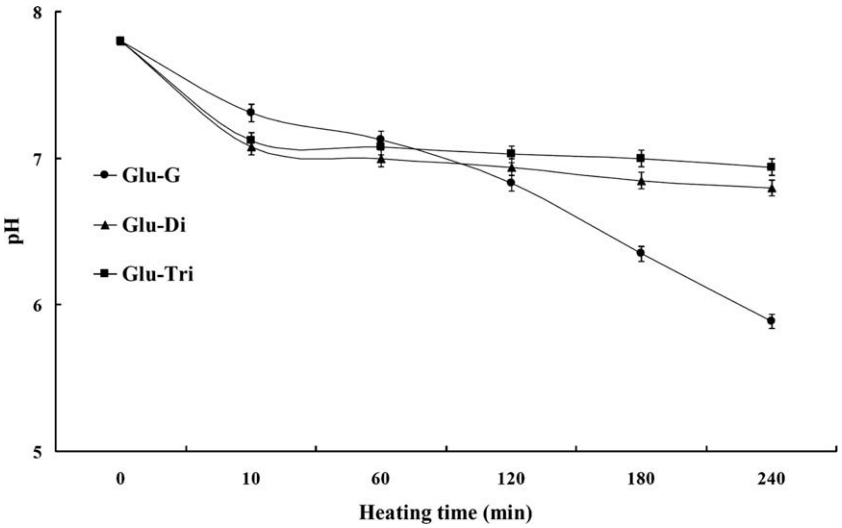

Fig. 1. The changes in pH of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time. Bars indicate the standard deviation from triplicate determinations.

heating time, are shown in Fig. 1. The pH of MRPs derived from the Glu-Di and Glu-Tri model systems with the same initial pH decreased markedly from their initial values within the first 10 min. Thereafter, the pH decreased slightly until 240 min of the heating time ($P < 0.05$). However, the pH of MRPs derived from the Glu-G model system decreased gradually as the heating time increased up to 240 min. Among the peptides tested, glycine rendered a higher pH of MRPs than did diglycine and triglycine, up to 60 min of the heating time. After 60 min, triglycine rendered the highest pH of MRPs. The result was in accordance with the result of studies conducted by Morales and Jimenez-Perez (2001) and Benjakul, Lertittikul, and Bauer (2005). They found that, during the Maillard reaction, the pH frequently decreases as the heating time increases. In the Maillard reaction, the pH is reduced, due to the formation of organic acids, such as formic and acetic acid (Brands & Van Boekel, 2002). Ogura, Nakayama, Nakaoka, and Nishihata (2000) reported that formation of the acid form of glycine, or glycyl–glycine, was due to the lowering of pH, which occurred, because oxygen evolution occurs in further positive polarisation. Huber and Wächtershäuser (1998) reported that the lowering of the pH can be explained by the formation of acids from CO during the heating process of peptides. In addition, some authors have reported that, during the reaction of peptides with carbonyl compounds, peptide bond breaking occurs through an anionic reaction (Chuyen, Kurata, & Fujimaki, 1973). However, for pH values above the isoelectric point of the amino compound (5.57 for glycine, 5.59 for diglycine and 5.58 for triglycine), the concentration of the anionic form is not a limiting factor (Reynols, 1969, chap. 12). Besides, it is expected that the molecule size could play a role in the smaller reactivity of peptides as compared to glycine (Buera, Chirife, Resnik, & Lozano, 1987). Therefore, the result suggested that the difference in the pH of MRPs was due to hydrolysis of the peptide bond and molecule size of peptides.

3.2. Changes in absorbance at 294 nm and browning intensity

The changes in absorbance at 294 nm and browning intensity of the MRPs derived from aqueous glucose/glycine, diglycine and triglycine model systems, as a function of the heating time, are shown in Fig. 2. The absorbance at 294 nm was used to determine the intermediate compounds of the Maillard reaction (Lerici, Barbanti, Manzano, & Cherubin, 1990). From the result, a sharp increase in A_{294} of all MRP samples was observed for up to 240 min of the heating time ($P < 0.05$). The MRPs derived from the Glu-Di model systems showed the highest increase in absorbance at 294 nm, followed by those derived from the Glu-Tri and Glu-G model systems. From the result, the increase in absorbance at 294 nm suggested the formation of an uncoloured compound, which could be the precursor of the Maillard reaction. This result was in agreement with that of Lerici et al. (1990), who found that the heat-treatment of a glucose–glycine mixture caused a marked increase in absorbance at 294 nm. The result suggested that intermediate products were produced, to a large extent, as the heating time increased. Moreover, the higher reactivity of triglycine than diglycine in the formation of intermediate compounds might be related to the availability of free glycine during the reaction.

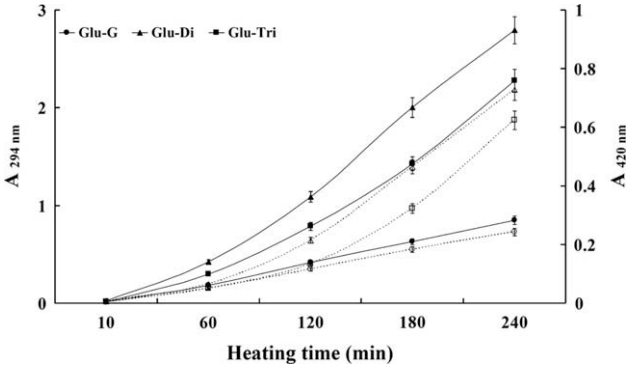

Fig. 2. The changes in absorbance at 294 nm and browning intensity of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time. Bars indicate the standard deviation from triplicate determinations (solid line: absorbance at 294 nm, dotted line: browning intensity).

Brown colour development (A 420 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 & Jimenez-Perez, 2001). From the result, the browning intensities of all MRP samples increased with a concomitant increase in A_{294}. A sharp increase in A_{420} of all MRP samples was observed for up to 240 min of the heating time ($P < 0.05$). The MRPs derived from the Glu-Di model system showed the highest increase in absorbance at 420 nm, followed by those of the Glu-Tri and Glu-G model systems.

This result was in agreement with that of Lu, Hao, Payne, and Ho (2005), who reported that diglycine–glucose reaction mixtures had higher degrees of browning, followed by glycine–glucose, and triglycine–glucose reaction mixtures. In addition, the UV absorbing and colourless compounds formed at the intermediate stages contributed to the brown pigment formation in both the Maillard and the caramelisation reactions (Benjakul et al., 2005). The result suggested that intermediate products and brown pigment had similar tendencies as the heating time increased. Moreover, the degree of browning might be dependent on a hydrolysis of the peptide bond. This result indicated that the stability of the peptide bond in triglycine was higher than that in diglycine.

3.3. Loss and enolisation of glucose

Monitoring sugar consumption is another way to evaluate the degree of reactivity of sugars in MRPs (Brands & Van Boekel, 2002; Lertittikul, Benjakul, & Tanaka, 2007). The enolisation reaction, known as the "Lobry de Bruyn–Alberda van Ekenstein transformation," produces enediol anion species. Glucose and fructose can isomerise into one another during this transformation (Speck, 1958). The loss and enolisation of glucose in the MRPs derived from aqueous glucose/glycine, diglycine and triglycine model systems, as a function of the heating time, are shown in Fig. 3. From the results, a sharp decrease in glucose of all MRP samples was observed for up to 240 min of the heating time ($P < 0.05$). The loss of glucose in MRPs derived from the Glu-G model system was lower than that in MRPs derived from the Glu-Di and Glu-Tri model systems. In particular, the losses of glucose in MRPs derived from the Glu-Di and Glu-Tri model systems were markedly decreased as the heating time increased and there was no statistical significance between the Glu-Di and the Glu-Tri model systems. In fact, little attention has been paid, so far, to the behaviour of the reducing sugar in studies on the Maillard reaction, although some authors, using mixtures of amino acids or proteins and reducing sugars, have reported that the carbonyl disappeared faster than its counterpart, the amino group (Ajandouz et al., 2001; Brands & Van Boekel, 2001). This observation was in agreement with the present study (Fig 3 and Table 1). In particular, De Kok and Rosing (1994) reported that sugar degradation, in the absence of peptide, was negligible, as was the hydrolysis of peptide in the absence of glucose. The reactivity in terms of glucose degradation was found to decrease in the order diglycine > triglycine > glycine, even though the pK_2 values were 8.25, 7.91, and 9.77, respectively, showing that the terminal amino group is not the only factor operating. Vernin et al. (1992) suggested that the fast disappearance of glucose may be due to the formation of diglycosylamines, in line with the findings of Lee, Sherr, and Koh (1984), who reported that difructosyl-lysine accounted for more than one quarter of the blocked lysine when a glucose–lysine mixture was refluxed for several hours in 80% methanol. The question that still remains to be answered, however, is what the effects of the peptide may be on the first chemical changes, which the reducing sugar undergo.

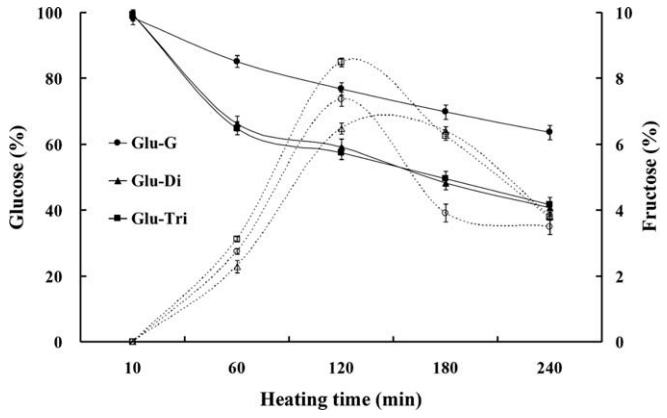

Fig. 3. The loss and enolisation of glucose in the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time. Bars indicate the standard deviation from triplicate determinations (solid line: glucose, dotted line: fructose).

On the other hand, the degree of sugar enolisation increased for up to 120 min of the heating time ($P < 0.05$). The degree of sugar enolisation in MRPs derived from the Glu-Tri model system was the highest, followed by those of the Glu-G and Glu-Di model systems. Thereafter, the degree of sugar enolisation, in all MRP samples, was decreased. The greatest decrease in the degree of sugar enolisation was observed in MRPs derived from the Glu-G model system. According to the Lobry de Bruyn–Alberda van Eckenstein rearrangement, all sugars, glucose and fructose are in equilibrium with the same intermediate, the 1,2-enediol. However, fructose is also in equilibrium with the 2,3-enediol. The formation of 1,2-enediol from the respective enaminol is not so likely to happen. However, by release of the amino acid the enaminol can form its 2,3-enediol, which through enolisation can lead to sugar formation, in particular fructose (Anet, 1964). This indicates that fructose can be formed from DFG (*N*-(1-deoxy-D-fructos-1-yl)-glycine) by its 2,3-enolisation step, whereas glucose can only be formed via the 1,2-enaminol, through the Schiff base in the Maillard reaction. Moreover, it could also be argued that the sugars might arise by aldol-type condensations between smaller sugar fragments generated from the decomposition of DFG (Sara & Van Boekel, 2003). A sugar isomerisation and degradation reaction also takes place, at the same time, during the course of the Maillard reaction. Sugar isomerisation and degradation reactions were reported to be much more important, from a quantitative point of view, than what is necessary in the Maillard reaction (Van Boekel, 1996). Because these sugar reactions occur simultaneously with the Maillard reaction and the sugar reaction products subsequently take part in the Maillard reaction, the Maillard reaction becomes even more intricate. Furthermore, conclusive evidence for the formation of sugar isomerisation during the course of the Maillard reaction has recently been found (Kim & Lee, 2008). Thus, sugar isomerisation can also occur during the course of the Maillard reaction with a peptide. The results suggest that the reducing sugar used as a reactant in the Maillard reaction, and the reaction rate, might be related to the degree of hydrolysis of the peptide bond.

3.4. Changes in glycine, diglycine and triglycine

At an early stage of the MRP formation, terminal α-amino groups of peptides react with the carbonyl function of reducing sugars present in the reaction medium. Thus, the loss of available primary amino groups is another indicator used to compare the sugar reactivity in the MRP (Benjakul et al., 2005; Lertittikul et al., 2007; Sun, Hayakawa, Puangmanee, & Izumori, 2006). The changes in glycine, diglycine and triglycine of the MRPs derived from aqueous glucose/glycine, diglycine and triglycine model systems, as a function of the heating time, are shown in Table 1. The stabilities of the two peptides can be determined by the remaining amount of peptides in the Maillard reaction. The glycine oligopeptides content of MRPs changed slightly when the heating time was 10 min. However, a continuous decrease in the glycine oligopeptides content of all MRP samples was observed when the heating time was increased ($P < 0.05$). The glycine oligopeptide content, in MRPs derived from the Glu-Di model system, was markedly decreased, followed by those of the Glu-Tri and Glu-G model systems. After 240 min of heating time, glycine remained at 65.2% (mol/mol%). Diglycine and triglycine remained between 49.1% and 53.7%

Table 1
The changes of glycine, diglycine, and triglycine (mol/mol%) in the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time.[a]

		Heating time (min)				
		10	60	120	180	240
Glu-G[c]	Glycine	98.6 ± 0.67[a]	86.5 ± 0.60[b]	80.3 ± 1.33[c]	78.4 ± 2.55[d]	65.2 ± 2.96[e]
	Diglycine	nd[b]	nd	nd	nd	nd
	Triglycine	nd	nd	nd	nd	nd
Glu-Di[d]	Glycine	nd	1.72 ± 0.08[d]	2.49 ± 0.17[c]	4.54 ± 0.10[b]	4.75 ± 0.06[a]
	Diglycine	98.3 ± 0.61[a]	64.7 ± 0.62[b]	62.3 ± 1.60[c]	56.3 ± 1.34[d]	49.1 ± 0.59[e]
	Triglycine	nd	nd	nd	nd	nd
Glu-Tri[e]	Glycine	nd	9.08 ± 0.33[d]	14.3 ± 0.56[c]	29.5 ± 0.37[a]	26.8 ± 0.70[b]
	Diglycine	1.24 ± 0.30[e]	2.22 ± 0.04[d]	2.64 ± 0.09[c]	4.28 ± 0.21[a]	3.49 ± 0.07[b]
	Triglycine	99.3 ± 0.10[a]	81.8 ± 0.79[b]	75.5 ± 2.41[c]	72.3 ± 0.96[d]	53.7 ± 2.03[e]

Different superscripts in the same row indicate significant differences ($P < 0.05$).
[a] Values represent mean ± SD ($n = 3$).
[b] nd, not detected.
[c] Glu-G, glycine with glucose.
[d] Glu-Di, diglycine with glucose.
[e] Glu-Tri, triglycine with glucose.

(mol/mol%) in the Glu-Di and Glu-Tri model systems. During the heating process, triglycine was mainly degraded into diglycine at about a 1.24–4.28% (mol/mol) yield and glycine at about a 9.08–29.5% (mol/mol) yield. Diglycine was degraded into glycine at about a 1.72–4.75% (mol/mol) yield. It was found that the Glu-Di model system generated less glycine than did the Glu-Tri model system, and the reason was considered to be the high electron density of the peptide bond, which suppresses hydrolysis. In addition, diglycine and triglycine were not detected in the Glu-G model system. Triglycine was not detected in the Glu-Di model system. The fact that glycine and diglycine were synthesised, with no detectable amount of diglycine or triglycine in the model systems, suggests that diglycine or triglycine molecules could be rapidly hydrolysed into molecules of glycine or diglycine. However, Nagayama, Takaoka, Inomata, and Yamagata (1990) reported that the possible mechanisms of formation of triglycine are chain elongation or opening of the diketopiperazine (DKP) ring with addition of one glycine molecule. Imai, Honda, Hatori, Brack, and Matsuno (1999) reported that, with repeated circulation of glycine through the hot (200–250 °C) and cold (0 °C) regions in a flow reactor at 24.0 MPa, oligopeptides were made from glycine. Therefore, this result suggested that the formation of an oligomer from glycine might be dependent on the extreme temperature and pressure conditions, as well as on DKP formation. Moreover, this result suggested that an α-NH_2 group of amino acids or proteins covalently attached to sugar to form glycated proteins to a greater extent, particularly when the heating time was increased. The first glycation product or Schiff base rearranges to a more stable ketoamine or Amadori product. The Amadori products can then form cross-links, between adjacent proteins or with other amino groups, resulting in polymeric aggregates called advanced glycation end-products (Friedman, 1996). From the results, the decreases in amino groups were in accordance with the increase in browning and absorbance at 294 nm (Fig. 2). This indicated that extended heating catalysed the interaction between amino groups and sugar via the glycation process. As a result, intermediate products were derived and converted further to brown pigments, as observed by the increased absorbance at 420 nm.

3.5. Wavelength spectra of MRPs

The changes in the wavelength spectra of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time are shown in Fig. 4. The absorbance of all MRP samples increased markedly as the heating time increased for up to 240 min. All MRP samples showed different absorptions in the UV–vis spectra, although these had similar shapes. The higher intensity of all MRP samples was indicative of increased absorbance in the ultraviolet region, and the absorbance was gradually reduced in the visible region. Every peak had a maximum absorbance that appeared in the range 260–320 nm, which is characteristic of melanoidins. This trend was also described by other authors for melanoidin-type colourants (Kim & Lee, 2008; Rafik, Mas, Elharfi, & Schue, 1997). In particular, the band intensities of MRPs derived from the Glu-Di and Glu-Tri model systems were significantly higher than those of MRPs derived from the Glu-G model system as the heating time increased. This wavelength shift was supposedly due to a possible increase in the extent of chromophore conjugation in melanoidins, resulting in a copigmentation effect. UV–vis spectra, exhibiting both featureless end absorption and increased intensity with a decrease in the wavelength, were typical of melanoidins (Clark & Tannenbaum, 1970). Further investigations of heated protein/glucose mixtures revealed that browning is accompanied by polymerisation of the protein (Kato, Matsuda, Kato, Watanabe, & Nakamura, 1986). Ultraviolet–visible (UV–vis) radiation comprises only a small part of the electromagnetic spectrum (about 100–750 nm). In simplified terms, UV–vis absorption spectroscopy can be described as spectroscopy involving the electronic energy levels of the molecule. Hence, the absorption of radiation leads to transitions among the electronic energy levels of the molecule (Wiberg, 2004). Therefore, this result indicated that the MRPs derived from the Glu-G, Glu-Di, and Glu-Tri model systems had different electronic energy levels of the molecules as the heating time increased.

3.6. Evolution of MW in MRPs

A molecular weight calibration curve for the TSK-G3000PW gel filtration column was intended for the determination of the peaks for MRPs that have high MWs of over 1000 Da. The correlation performed revealed that the TSK gel filtration column could serve as a good tool to separate proteins in the range of 1000 Da–4000 kDa. The MW of the eluted proteins was calculated in the following equation:

$$y = 6.5726 - 0.1654x \ (R^2 = 0.987)$$

where y = log MW, and x is the retention time.

The changes in the gel permeation chromatograms of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems, as a function of the heating time, are shown in

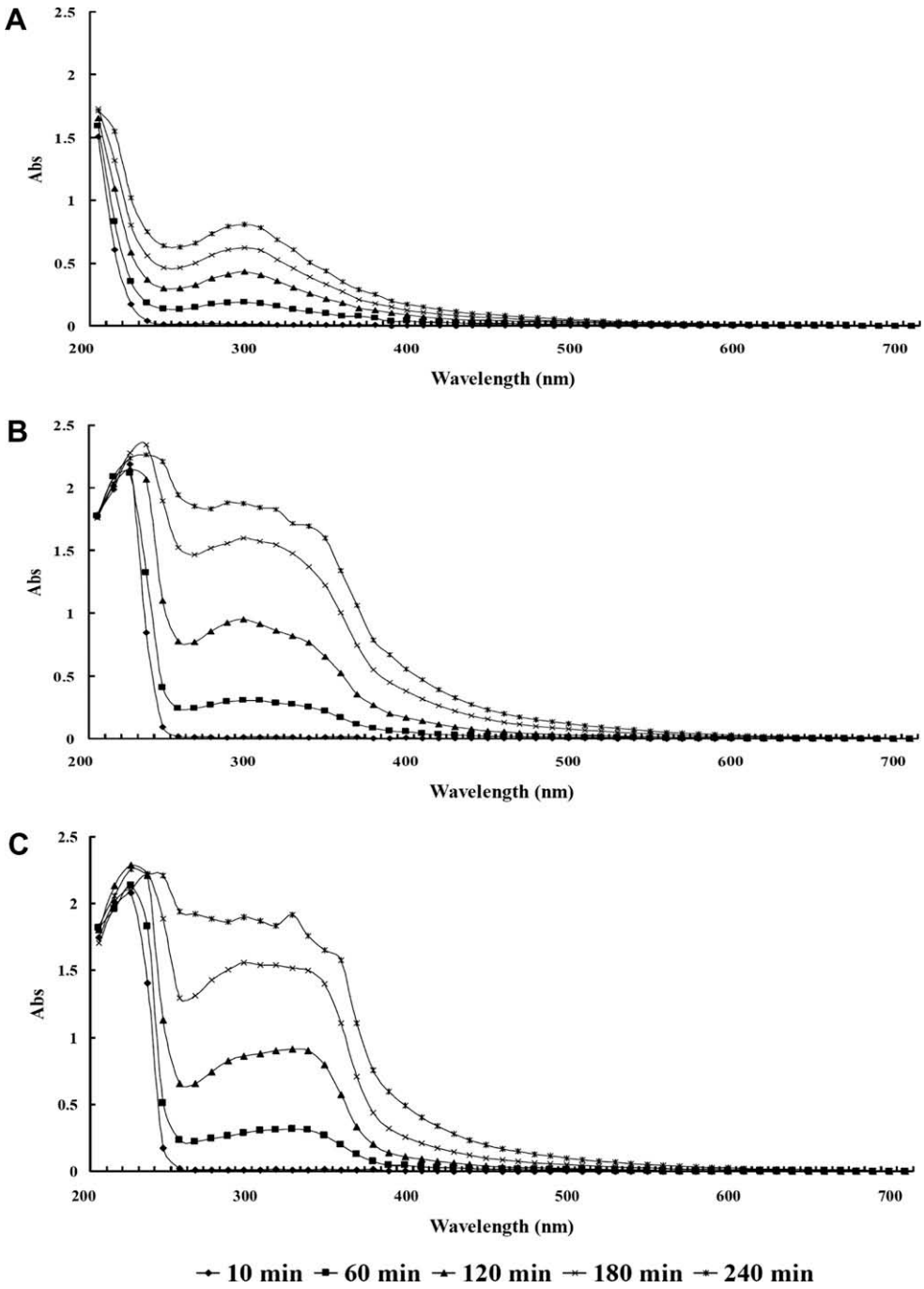

Fig. 4. The changes in the wavelength spectra of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time.

Fig. 5. Gel permeation chromatography (GPC) allowed separation of MRPs on a MW basis. Using this technique, low-molecular-weight (LMW) compounds were retained in the pores of gel beads and eluted later than were the molecules of HMW compounds. In the present study, the term "MRP" is arbitrarily used for all copolymers with a molecular weight above 5000 Da. In addition, the molecular weight of all MRP samples was less than 40 kDa, as they were compared with MW calibration standards. As the heating time increased, the MW of all MRP samples showed rising intensities, whereas the major peaks of each MRP sample were eluted at different retention times. With regard to the molecular size distribution of MRPs derived from the Glu-G model system (Fig. 5A), four peaks with different molecular masses were observed, corresponding to the peak apices. The major peaks, appearing at 15.14 and 15.86 min, corresponded to molecular weights of 11.6 and 8.9 kDa, respectively. The other peaks, with retention times of 17.22 and 20.76 min, corresponded to molecular weights of 5.4 and 1.4 kDa, respectively. In particular, compounds with MWs of 11.6 and 8.9 kDa increased markedly as the heating time increased up to 240 min. On the other hand, with regard to the MRPs derived from the Glu-Di model system, the chromatograms shown in Fig. 5B confirmed that the shape of the chromatogram increased more than did those of MRPs derived from the Glu-G model system. Four peaks, with different molecular masses, were also observed. The first peak, appearing at 14.63 min, corresponded to a molecular weight of 14.2 kDa. The second peak, with a retention time of 16.63 min, corresponded to a molecular weight of 6.6 kDa. The third peak, with a retention time of 17.36 min, corre-

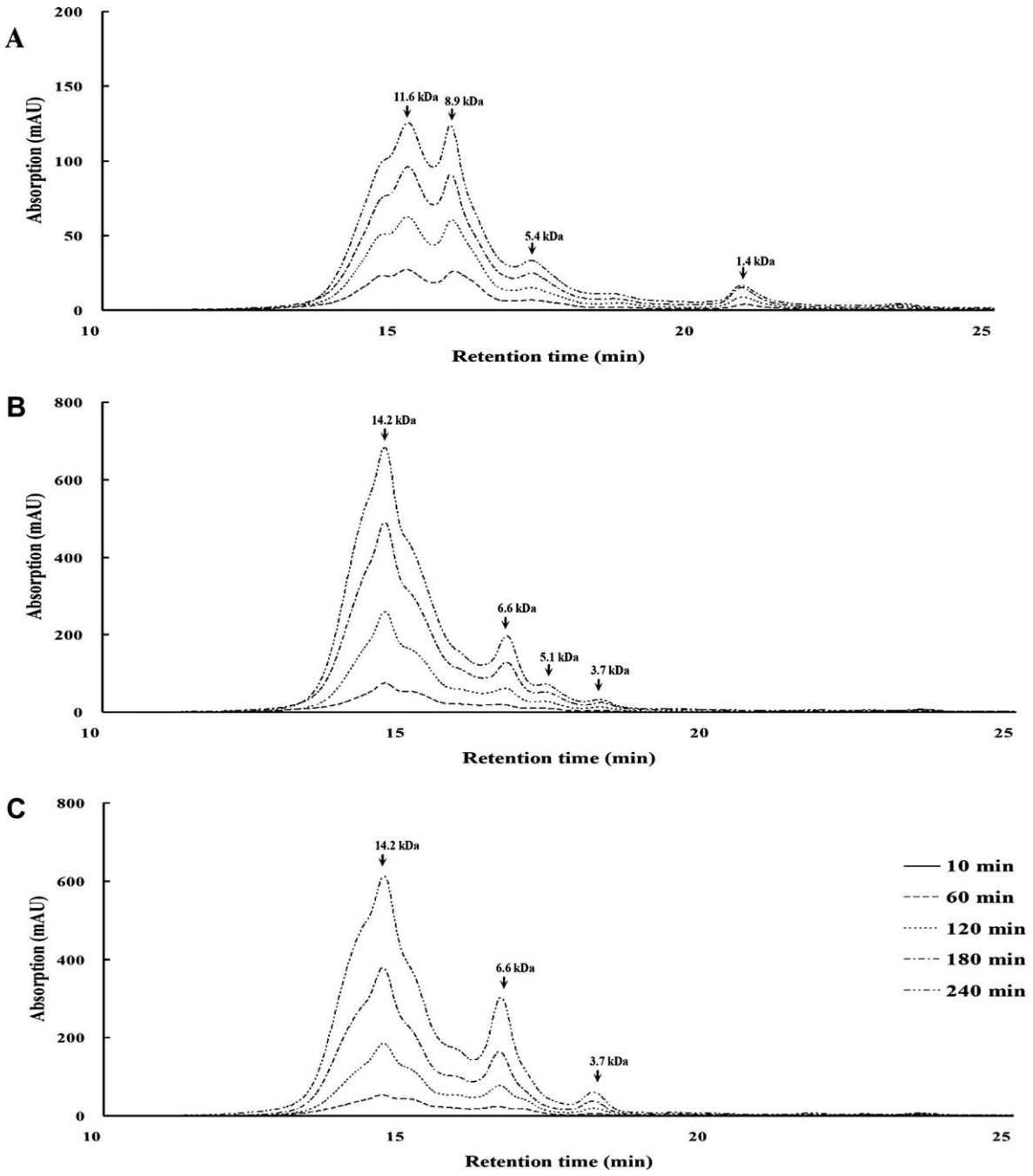

Fig. 5. GPC chromatography patterns of the MRPs derived from aqueous glucose/glycine, diglycine, and triglycine model systems as a function of the heating time.

sponded to a molecular weight of 5.1 kDa. The last peak, with a retention time of 18.16 min, corresponded to a molecular weight of 3.7 kDa. In particular, the compounds with a MW of 14.2 kDa increased markedly as the heating time increased up to 240 min. Moreover, with regard to the molecular size distribution of MRPs derived from the Glu-Tri model system (Fig. 5C), three peaks were observed with different molecular masses, corresponding to the peak apices. The three peaks had quite similar retention times, and corresponded to MRPs derived from the Glu-Di model system. The major peak, appearing at 14.63 min, corresponded to a molecular weight of 14.2 kDa. The other peaks, with retention times of 16.63 and 18.16 min, corresponded to molecular weights of 6.6 and 3.7 kDa, respectively. In particular, the compounds with MWs of 14.2 and 6.6 kDa increased markedly as the heating time increased. The shape of chromatogram with a MW of 14.2 changed slightly more than did those of MRPs derived from the Glu-Di model system, while the peak with a MW of 6.6 kDa increased. Hofmann (1998) reported that the reaction between casein and glucose led to a drastic increase in the MWs by carbohydrate-induced oligomerisation of the protein backbone. Furthermore, these results confirm the earlier findings of Clark and Tannenbaum (1974) that the reaction between proteins and carbohydrates leads to polymerisation of the reaction mixture. The results showed that the shapes of the chromatogram of MRPs derived from the Glu-Di and Glu-Tri model systems were different from those of MRPs derived from the Glu-G model system as the heating time increased. Moreover, the MRPs of different peptides had different SEC elution patterns. It should be noted that the ratio between the peak areas changed significantly as the heating time increased. There is evidence that the shape of the peaks of the MRPs was independent

of temperature but dependent on the type of peptide. This fact suggests that the SEC elution pattern can be considered a reliable "structure fingerprinting" of the MRPs.

4. Conclusion

The results have shown that the Maillard reaction is greatly affected by the peptide chain length. The stability of the peptide bond in triglycine was higher than that in diglycine. Thus, the Maillard reaction rate might be related to the degree of hydrolysis of the peptide bond and the stability of the peptide bond as the heating time increased. Furthermore, these results suggested that the peptide chain length may be one of the main factors affecting MRP formation via the Maillard reaction.

References

Aaslyng, M. D., Martens, M., Poll, L., Nielsen, P. M., Flyge, H., & Larsen, L. M. (1998). Chemical and sensory characterization of hydrolyzed vegetable protein, a savory flavoring. *Journal of Agricultural and Food Chemistry, 46*, 481–489.

Ajandouz, E. H., Tchiakpe, L. S., Ore, F. D., Benajiba, A., & Puigserver, A. (2001). Effects of pH on caramelization and Maillard reaction kinetics in fructose–lysine model systems. *Journal of Food Science, 66*, 926–931.

Anet, E. F. L. J. (1964). 3-Deoxyglycosuloses (3-deoxyglycosones) and the degradation of carbohydrates. *Advances in Carbohydrate Chemistry and Biochemistry, 19*, 181–218.

Benjakul, S., Lertittikul, W., & Bauer, F. (2005). Antioxidant activity of Maillard reaction products from a porcine plasma protein–sugar model system. *Food Chemistry, 93*, 189–196.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2001). Reactions of monosaccharides during heating of sugar–casein systems: Building of a reaction network model. *Journal of Agriculture and Food Chemistry, 49*, 4667–4675.

Brands, C. M. J., & Van Boekel, M. A. J. S. (2002). Kinetics modeling of reactions in heated monosaccharide–casein system. *Journal of Agricultural and Food Chemistry, 50*, 6725–6739.

Budavari, S., O'Neil, M. J., Smith, A., Heckelman, P. E., & Kinneary, J. F. (1996). *The Merck index* (12th ed.). Whitehouse Station, NY: Merck & Co. pp. 1477–1478.

Buera, M. D. P., Chirife, J., Resnik, S. L., & Lozano, R. D. (1987). Nonenzymatic browning in liquid model systems of high water activity: Kinetics of color changes due to reaction between glucose and glycine peptides. *Journal of Food Science, 52*, 1059–1062.

Chawla, S. P., Chander, R., & Sharma, A. (2007). Antioxidant formation by γ-irradiation of glucose–amino acid model systems. *Food Chemistry, 103*, 1297–1304.

Chuyen, N. V., Kurata, T., & Fujimaki, M. (1973). Studies on the reaction of dipeptides with glyoxal. *Agricultural and Biological Chemistry, 37*, 327–334.

Clark, A. V., & Tannenbaum, S. R. (1970). Isolation and characterization of pigments from protein-carbonyl browning systems. Isolation, purification, and properties. *Journal of Agricultural and Food Chemistry, 18*, 891–894.

Clark, A. V., & Tannenbaum, S. R. (1974). Isolation and characterization of pigments from protein-carbonyl browning systems. Models for two insulin–glucose pigments. *Journal of Agricultural and Food Chemistry, 22*, 1089–1093.

De Kok, P. M. T., & Rosing, E. A. E. (1994). Reactivity of peptides in Maillard reaction. In T. H. Parliment, M. J. Morello, & R. J. McGomn (Eds.), *Thermally generated flavors: Maillard, microwave, and extrusion processes* (pp. 158–179). Washington: American Chemical Society.

Friedman, M. (1996). Food browning and its prevention: An overview. *Journal of Agricultural and Food Chemistry, 44*, 631–653.

Hartman, R., & Meisel, H. (2007). Food-derived peptides with biological activity: From research to food applications. *Current Opinion in Biotechnology, 18*, 163–169.

Hofmann, T. (1998). Studies on the relationship between molecular weight and the color potency of fractions obtained by thermal treatment of glucose/amino acid and glucose/protein solutions by using ultracentrifugation and color dilution techniques. *Journal of Agriculture and Food Chemistry, 46*, 3891–3895.

Huber, C., & Wächtershäuser, G. (1998). Peptides by activation of amino acids with CO on (Ni, Fe)S surfaces: Implications for the origin of life. *Science, 281*, 670–671.

Imai, E., Honda, H., Hatori, K., Brack, A., & Matsuno, K. (1999). Elongation of oligopeptides in a simulated submarine hydrothermal system. *Science, 283*, 831–833.

Kato, Y., Matsuda, T., Kato, N., Watanabe, K., & Nakamura, R. (1986). Browning and insolubilization of ovalbumin by the Maillard reaction with some aldohexoses. *Journal of Agriculture and Food Chemistry, 34*, 351–355.

Kim, J. S., & Lee, Y. S. (2008). Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as result of the Maillard reaction. *Food Chemistry, 108*, 582–592.

Lee, C. M., Sherr, B., & Koh, Y. N. (1984). Evaluation of kinetic parameters for a glucose–lysine Maillard reaction. *Journal of Agriculture and Food Chemistry, 32*, 379–382.

Lerici, C. R., Barbanti, D., Manzano, M., & Cherubin, S. (1990). Early indicators of chemical changes in foods due to enzymic or non enzymic browning reactions. 1: Study on heat treated model systems. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 23*, 289–294.

Lertittikul, W., Benjakul, S., & Tanaka, M. (2007). Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chemistry, 100*, 669–677.

Lu, C. Y., Hao, Z., Payne, R., & Ho, C. T. (2005). Effects of water content on volatile generation and peptide degradation in the Maillard reaction of glycine, diglycine, and triglycine. *Journal of Agriculture and Food Chemistry, 53*, 6443–6447.

Ludwig, E., Lipke, U., Raczek, U., & Jäger, A. (2000). Investigation of peptides and proteases in green coffee beans. *European Food Research Technology, 211*, 111–116.

Martins, S. I. F. S., & Van Boekel, M. A. J. S. (2005a). A kinetic model for the glucose/glycine Maillard reaction pathway. *Food Chemistry, 90*, 257–269.

Martins, S. I. F. S., & Van Boekel, M. A. J. S. (2005b). Kinetics of the glucose/glycine Maillard reaction pathways: Influences of pH and reactant initial concentrations. *Food Chemistry, 92*, 437–448.

Morales, F. J., & Jimenez-Perez, S. (2001). Free radical scavenging capacity of Maillard reaction products as related to colour and fluorescence. *Food Chemistry, 72*, 119–125.

Nagayama, M., Takaoka, O., Inomata, K., & Yamagata, Y. (1990). Diketopiperazine-mediated peptide formation in aqueous solution. *Origins of Life and Evolution of the Biosphere, 20*, 249–257.

Naranjo, G. B., Malec, L. S., & Vigo, M. S. (1998). Reducing sugars effect on available lysine loss of casein by moderate heat treatment. *Food Chemistry, 62*, 309–313.

Ogura, K., Nakayama, M., Nakaoka, K., & Nishihata, Y. (2000). Spectroelectrochemical and EQCM studies on the oxidation of glycil-peptides in alkaline medium. *Journal of Electroanalytical Chemistry, 482*, 32–39.

Oh, Y. C., Hartman, T. G., & Ho, C. T. (1992). Volatile compounds generated from the Maillard reaction of Pro-Gly, Gly-Pro, and a mixture of glycine and proline with glucose. *Journal of Agriculture and Food Chemistry, 40*, 1878–1880.

Oh, Y. C., Shu, C. K., & Ho, C. T. (1991). Some volatiles compounds formed from thermal interaction of glucose with glycine, diglycine, triglycine, and tetraglycine. *Journal of Agriculture and Food Chemistry, 39*, 1553–1554.

Rafik, M., Mas, A., Elharfi, A., & Schue, F. (1997). Decoloration de solutions sucrees par ultrafiltration sur une membrane a base de poly (organocyclophosphazene). *European Polymer Journal, 33l*, 679–690.

Reynols, T. M. (1969). Non-enzymatic browning. Sugar–amine interaction. In H. W. Shultz (Ed.), *Carbohydrates and their roles*. Westport, CT: AVI Pub. Co.

Rizzi, G. P. (1989). Heat-induced flavor from peptides. In T. H. Parliment, R. J. McGorrin, & C. T. Ho (Eds.). *Thermal generation of aromas, ACS symposium series* (Vol. 409, pp. 285–301). Washington: American Chemical Society.

Sara, I. F. S. M., & Van Boekel, M. A. J. S. (2003). Melanoidins extinction coefficient in the glucose/glycine Maillard reaction. *Food Chemistry, 83*, 135–142.

Speck, J. C. J. (1958). The Lobry de Bruyn–Alberda van Ekenstein transformation. *Advances in Carbocation Chemistry, 13*, 63–103.

Sun, Y., Hayakawa, S., Puangmanee, S., & Izumori, K. (2006). Chemical properties and antioxidative activity of glycated α-lactalbumin with a rare sugar, D-allose, by Maillard reaction. *Food Chemistry, 95*, 509–517.

Taborda, G., Gómez-Riuz, J. A., Martínez-Castro, I., Amigo, L., Ramos, M., & Molina, E. (2008). Taste and flavor of artisanal and industrial Manchego cheese as influenced by the WSN compounds. *European Food Research Technology, 227*, 323–330.

Van Boekel, M. A. J. S. (1996). Kinetic modelling of sugar reactions in heated milk-like systems. *Netherlands Milk and Dairy Journal, 50*, 245–266.

Vernin, G., Metzger, J., Boniface, C., Murello, M. H., Siouffi, A., Larice, J. L., et al. (1992). Kinetics and thermal degradation of the fructose–methionine Amadori intermediates. GC–MS/SPECMA data bank identification of volatile aroma compounds. *Carbohydrate Research, 230*, 15–29.

Wiberg, K. (2004). Multivariate spectroscopic methods for the analysis of solutions. PhD thesis, Stockholm University, The Sweden.

Wijewickreme, A. N., Kitts, D. D., & Durance, T. D. (1997). Reaction conditions influence the elementary composition and metal chelating affinity of nondialyzable model Maillard reaction products. *Journal of Agricultural and Food Chemistry, 45*, 4577–4583.