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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

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Abstract

This study investigates the effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers, can influence the formation of melanoidins as result of the Maillard reaction. Remarkable enolization reaction of sugars was observed in the course of the Maillard reaction. Especially, the degree of sugar enolization was increased as the pH levels increased, which was higher in fructose than glucose systems. Otherwise, enolization of sugars on heating with amino acid was higher in glucose than fructose systems. Formation of isomer in Glc/D-Lys, Fru/D-Asn and Fru/D-Lys were increased upon increase of pH levels. The relative amounts of isomers in Glc/L-Asn and Glc/D-Asn were decreased upon increase of pH levels. Browning development was dependent on the pH levels, being more significant for model systems apart from heated glucose solution alone. Browning development of fructose systems was higher than glucose-amino acid systems. The L- and D-isomers both showed different absorption in the UV–vis spectra and that these occur at similar shape. Every peak has a stable absorbance appeared in the range between 260 and 320 nm, characteristic of melanoidins.

Keywords: Amino acid enantiomers; Enolization; Maillard reaction; Melanoidins; Racemization

1. Introduction

The reaction between reducing sugars and amino acids is known as the Maillard reaction or non-enzymic browning reaction (Maillard, 1913). 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).

Enolization reaction known as the "Lobry de Bruyn–Alberda van Ekenstein transformation" produces enediol anion species. Through the reactions of sugar transformation due to the Lobry de Bruyn–Alberda van Ekenstein transformation, aldoses are converted into ketoses and vice versa. Similarly, an aminoketose is converted into aminoaldose in the presence of the free amino acids. Sugar isomerization and degradation reactions were reported to be much more important from a quantitative point of view than the Maillard reaction (Berg & Van Boekel, 1994; 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.

The conversion of free or protein- or peptide-bound physiological L-amino acids into their mirror images (enantiomers) named D-amino acids is of great interest from the nutritional and physiological point of view (Friedman, 1999). This process of the change of chirality ("handedness") of amino acids is commonly referred to as racemization or epimerisation if several chiral centers are involved, although, in the strict sense, racemic amino acids contain

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equal amounts of D- and L-amino acids (Brückner, Justus, & Kirschbaum, 2001). The Maillard reaction can also explain the formation of D-amino acids in food. Brückner et al. (2001) have recently pointed out that D-amino acids are formed on heating aqueous solutions of L-amino acids (2.5 mM) together with an excess (278 mM) of saccharides (glucose, fructose, and saccharose) at 100 °C for 24–96 h in aqueous solutions of pH 2.5 (AcOH) or pH 7.0 (NaOAc). Thus, the formation of D-amino acids in many foods of plant and animal origin is the result of nonenzymic browning since the presence of amino acids together with saccharides is common. As for the racemization mechanism, it is postulated that the reaction of amino acids with glucose or fructose starts with the reversible formation of Schiff bases. The degree of racemization depends in particular on steric and electronic properties of the amino acid side chains. It should be noted that the early stages of the Maillard reaction proceeds already under mild conditions (Brückner et al., 2001; Ledl & Schleicher, 1990) and do not require alkaline or acidic condition. This new racemization mechanism based on the relatively stable Amadori compounds has been used to explain the generation of free D-amino acid in foods such as dried fruits, concentrated plant juices and fortified wines (Pätzold, Nieto-Rodriguez, & Brückner, 2003). Recently, heating experiments of synthetic Amadori compounds proved that they are sources of amino acid enantiomers (Pätzold & Brückner, 2005, 2006a, 2006b). Amino acid racemization, however, is very much dependent on temperature, pH, and presence of catalysts (Bada, 1972). Furthermore, convincing evidence has recently been established that D-amino acids are formed in the course of the Maillard reaction (Brückner et al., 2001; Pätzold & Brückner 2005, 2006a).

There have been two basic approaches for the chromatographic resolution of enantiomers of amino acids: a direct and an indirect method (Bhushan & Brückner, 2004). The direct approach requires no chemical derivatization prior to separation process. Resolution is possible through reversible diastereomeric association between the chromatographic chiral environment and the solute enantiomers. Direct methods have certain critical disadvantages. Protein stationary phases are not durable over time and pH and also have low sample capacity. Besides, the correct elution order is difficult to be predicted because of the complexity of interactions with the protein (Pirkle & Pochapsky, 1989). On the other hand, the separation of diastereomeric pair via the indirect method is sometimes simpler to perform and often has better resolution than with a direct method because chromatographic conditions are much easily optimized. There is little doubt that of the various methods available for the indirect HPLC resolution of enantiomers of amino acids use of Marfey's reagent has been most successful. Marfey's reagent provides a very simple and effective analytical method for the chromatographic resolution of enantiomers of amino acids. It has been widely used for structural characterization of peptides, confirmation of racemization in peptide synthesis, and detection of small quantities of D-amino acids. Marfey's method uses amino acid derivatization with 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA) to form diastereomers of amino acids.

From the point of view of color, melanoidins can be built up of subunits in two contrasting ways. One possibility is that melanoidins are formed by more or less random reaction of low molecular weight reaction intermediates (which may inherently be colored or not). Alternatively, a repeating unit (which may be colorless or contribute little to color) may form the backbone of melanoidins, which chromogenic low molecular weight structures attaching themselves to this backbone, resulting in high-molecular-weight colored structures. The development of color is an extremely important and obvious feature of the extent of the advanced Maillard reaction (Nursten, 1986). This stage characterized by the formation of unsaturated, brown nitrogenous polymers and copolymers, although nitrogen-free polymers are also formed from condensation reactions from furfurals or dehydroreductones (Ames, 1992, Chap. 4; Hodge, 1953). Ames and Nursten (1989) grouped the colored compounds into two general classes: low molecular weight compounds, which typically possess two-to-four linked rings containing extended double-bond conjugation (Ames, 1992, Chap. 4; Ames, Apriyantono, & Arnoldi, 1993; Ledl & Severin, 1978, 1982), and melanoidins which are brown polymers and possess molecular weights of several thousand daltons and discrete chromophore groups (Ames & Nursten, 1989; O'Brien and Morrissey, 1989).

The aim of the present study was to investigate the effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers, can influence the formation of melanoidins as result of the Maillard reaction. Melanoidins were, rather arbitrarily, defined as high-molecular-weight (HMW) by a lower limit of 3500 Da, which was the nominal cut-off value in the dialysis system used.

2. Materials and methods

2.1. Chemicals

D-Glucose, D-fructose, glycine, L-asparagine, D-asparagine, L-lysine and D-lysine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium carbonate and sodium hydrogen phosphate were purchased from Merck Co. (Darmstadt, Germany). HPLC-grade water was purchased from J.T. Baker (Phillipsburg, NJ, USA). Reagents were of highest reagent grade quality and used without any further purification.

2.2. Preparation of Maillard reaction products (MRPs)

Glucose, fructose and amino acids were dissolved in 100 ml of 0.5 M sodium acetate buffer, pH 4.0, 0.5 M phosphate buffer, pH 7.0 or 0.5 M sodium carbonate buffer, pH 10.0 to obtain a final concentration of 1 M. Twelve model

systems were prepared, being glucose (Glc), glucose–glycine (Glc/Gly), glucose–L-asparagine (Glc/L-Asn), glucose–D-asparagine (Glc/D-Asn), glucose–L-lysine (Glc/L-Lys), glucose–D-lysine (Glc/D-Lys), fructose (Fru), fructose–glycine (Fru/Gly), fructose–L-asparagine (Fru/L-Asn), fructose–D-asparagine (Fru/D-Asn), fructose–L-lysine (Fru/L-Lys) and fructose–D-lysine (Fru/D-Lys). The reaction mixtures were then distributed over glass, screw-capped, Schott tube (16×160 mm), each containing a minimum of 10 ml. Model solutions were heated without pH control in at least duplicated at 100 °C for 2 h. The heating was carried out in a silicone oil bath and the proper safety measures taken. After heated, model solutions were withdrawn, immediately cooled in ice water and then dialysed or undialysed.

2.3. Dialysis

Approximately 2 ml of the reaction mixture were injected into dialysis cassettes (Mr > 3500) (Slide-Alyzer Dialysis Cassette, 3.5K MWCO, Pierce, IL, USA) and dialyzed against distilled water. Batch dialysis was performed against 1500 ml double distilled water over 168 h at 4 °C. Water was changed every 3 h for 12 h and then every 10–12 h for the rest of the dialysis time. After dialysis, samples were freeze-dried and stored in a dessicator at 4 °C until analysis. MRPs samples before and after dialysis were dissolved in water before use.

2.4. Determination of sugars in MRPs

The reducing sugars in MRPs before dialysis were determined using 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, 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 portions were injected into the HPLC system. The data analysis was performed using Chemstation software (Hewlett Packard).

2.5. Derivatization of amino acids with FDAA in MRPs

Amino acids were derivatized with FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) reagent according to Marfey's methods (Marfey, 1984). Ten microlitres of MRPs before dialysis in 20 μl of H_2O and 8 μl of 1 mol/l $NaHCO_3$ were mixed with 400 μg of FDAA in 40 μl acetone and incubated at 40 °C for 1 h with occasional shaking. The reaction was terminated by adding 4 μl of 2 mol/l HCl. Acetone, water and HCl were removed by evaporation under reduced pressure in a centrifugal evaporator. After evaporation, 20 μl of methanol were added to dissolve the resultant FDAA amino acid. FDAA amino acid solution (2 μl), thus, prepared (0.5%, w/v) was spotted on a reversed phase precoated TLC plate (RP-18, $F_{254}S$, 5 cm × 10, from Merck, Darmstadt, Germany), and developed with acetonitrile/triethylamine-phosphate buffer (50 mM, pH 5.5) at 25/75 (v/v) in a pre-equilibrated glass chamber at 25 °C. The FDAA amino acid spots were yellow and visible. When the ascending solvent front neared the top margin, the plate was removed from the chamber and dried with a hair-drier. The TLC was completed in 20 min at 25 °C. A trial for quantitative analysis was made by varying the amount of DL-amino acid derivatives to the plate. The yellow spots were scraped off the plate after the chromatography, and extracted with methanol/water (1/1, v/v). Then FDAA amino acids (derivatized amino acids) were analyzed by HPLC. Since FDAA is sensitive to light, the FDAA amino acids were not exposed to light during all procedures.

2.6. Determination of amino acids in MRPs

The amino acids in MRPs before dialysis were analyzed using HP 1100 liquid chromatograph (Hewlett Packard, Wilmington, DE, 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, Palo Alto, CA, USA). A linear gradient profile of mobile phase, comprising 40 mM Na_2HPO_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, pre-column derivatization with *o*-phthalaldehyde (OPA) was used and 0.5 μl portions were injected into the HPLC system. The data analysis was performed using Chemstation software (Hewlett Packard, Wilmington, DE, USA). Relative quantities of amino acid enantiomers were calculated from peak areas of derivatives: $\%L = 100L/(D + L)$, $\%D = 100D/(D + L)$, where $\%L$ and $\%D$ represents relative amounts of L- and D-amino acids with regard to the sum of $(D + L)$ amino acids and D and L represent the peak areas of the respective enantiomer determined by HPLC.

2.7. Measurement of browning

Browning indices of MRPs samples before and after dialysis were recorded by their absorbance at 420 nm on a spectrophotometer (Shimadzu UV 160A, Shimadzu Co., Kyoto, Japan) using a 1 cm pathlength cell after appropriate dilution with distilled water.

2.8. Wavelength spectra of melanodins

Wavelength spectra of melanoidins were recorded by a UV–vis spectrophotometer (Shimadzu UV 160A, Shima-

dzu Co., Kyoto, Japan), with the wavelength ranging from 200 nm to 700 nm.

2.9. Statistical analysis

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

3. Results and discussion

3.1. The loss and enolization of sugar in MRPs

The loss and enolization of sugar in MRPs from glucose and fructose prepared at different pH levels are shown in Fig. 1. The concentration of the reactants was decreased according to increase of pH levels. Moreover, the loss of glucose was more than the loss of fructose, regardless of amino acid enantiomers. The sugars were almost completely destroyed in the MRPs resulting from L- and D-lysine. Enolization reaction known as the "Lobry de Bruyn–Alberda van Ekenstein transformation" produces enediol anion species. Glucose and fructose can isomerise into one another this transformation (Speck, 1958). In present study, the degree of sugar enolization was increased as increase of pH levels except for Glc, Fru, Glc/L-Lys, Glc/D-Lys, Fru/L-Lys and Fru/D-Lys. Especially, in Glc/L-Lys, Glc/D-Lys and Fru/D-Lys system, enolization of sugar was found at pH 4.0. The degree of sugar enolization was higher in Fru than Glc. Otherwise, enolization of sugars on heating with amino acid was higher in glucose than fructose systems. Enolization was not showed different characteristics, as amino acid enantiomers. Higher levels of degradation of both fructose and glucose occurred at 100 °C under alkaline conditions (Ajandouz & Puigserver, 1999; Ajandouz, Tchiakpe, DalleOre, Benajiba, & Puigserver, 2001; Yang & Montgomery, 1996). Benjakul, Visessanguan, Phongkanpai, and Tanaka (2005) also found that sugars were lost faster in alkaline solutions than in neutral solutions. 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-endiol. However, fructose is also in equilibrium with the 2,3-endiol. The formation of 1,2-endiol from the respective enaminol is not so likely to happen. However, by release of the amino acid the enaminol can from its 2,3-endiol, which through enolization can lead to sugars 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-enolization step, whereas mannose and 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). Thus, sugar isomerisation also can be formed in Maillard reaction by L- and D-amino acid. The results suggest that both glucose and fructose underwent degradation to a greater extent under the alkaline pH. The enolization might take place favorably at alkaline pH, leading to the transformation of those sugars.

3.2. The loss and racemization of amino acids in MRPs

The quantities of L- and D-amino acids determined by HPLC in MRPs and the relative quantities of their isomers are shown in Table 1. The concentration of the reactants was increased according to increase of pH levels except for Glc/D-Lys, Fru/L-Asn, Fru/D-Asn, Fru/L-Lys and Fru/D-Lys. Moreover, at pH 4.0 and 7.0, the quantities of L- and D-amino acids were much in fructose systems than glucose systems. However, at pH 10.0, glucose systems were much than fructose systems. This characteristic was found in all systems except for Glc/D-Lys and Fru/D-Lys. Especially, the quantity of amino acids in Glc/L-Lys was the lowest as increasing pH.

D-Amino acid was detected in L-amino acid systems. Similarly, L-amino acid could also be observed in D-amino acid systems. Formation of isomer in Glc/D-Lys, Fru/D-Asn and Fru/D-Lys were increased as increase of pH levels. As increasing pH levels, racemization of D-Asn was higher in fructose system, whereas L-Lys was higher in glucose system. The relative amounts of isomers in Glc/L-Asn and Glc/D-Asn were decreased as increase of pH levels. However, Fru/L-Lys and Fru/D-Lys were increased. In addition, Glc/L-Lys and Fru/L-Asn were decreased and then increased, while Glc/D-Lys and Fru/D-Asn were increased and then decreased according to pH level. Brückner et al. (2001) had previously shown that heating of L-amino acids together with reducing sugars leads to the formation of large amounts of D-amino acids. This reaction has been known as the Maillard reaction (Ledl & Schleicher, 1990) or non-enzymic browning reaction (Friedman, 1996). Pätzold and Brückner (2004) had postulated that D-amino acids are generated from relatively stable intermediates of this reaction, named the Amadori and Heyns compounds. Release of amino acids from Amadori compounds is reversible until the amino acids are finally transferred at advanced stages of the Maillard reaction irreversibly in heterocyclic or polymeric compounds. Consequently, generation of D-amino acid from Amadori compounds (or Heyns compounds resulting from fructose and amino acids) is postulated to be a major route for their formation in the Maillard reaction. A tentative mechanism via formation of a carbanion in the Amadori compound has been presented (Pätzold & Brückner, 2004). This general route for the generation of D-amino acids has been extended to other foods rich in reducing sugars and amino acids. The Maillard reaction also explains the occurrence of D-amino acids in roasted coffee and cacao (Casal, Mendes, Oliveira, & Ferreira, 2005; Kutz, Pätzold, & Brückner, 2004).

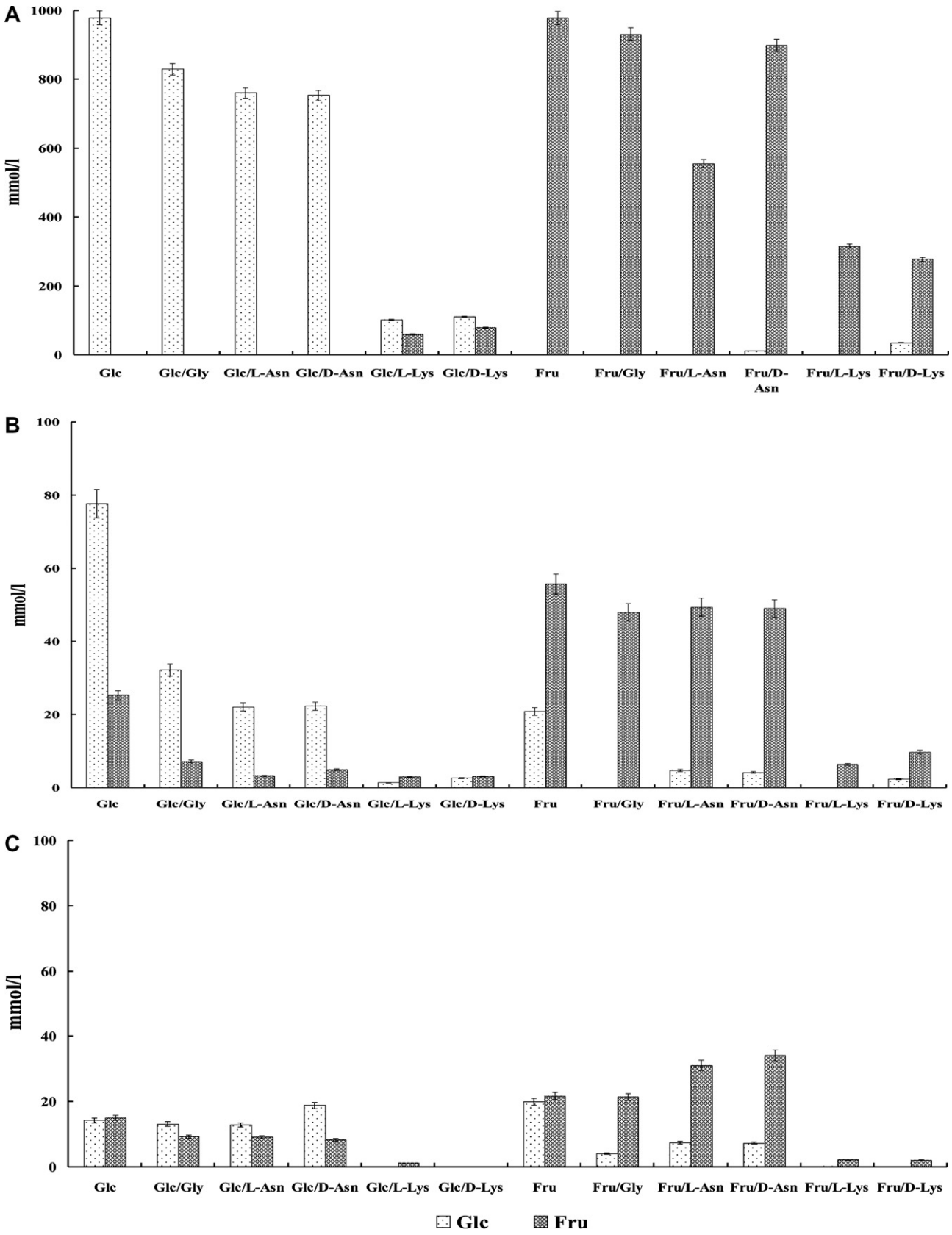

Fig. 1. The loss and enolization of sugars in MRPs from glucose and fructose prepared at different pH levels. (A) pH 4.0; (B) pH 7.0 and (C) pH 10.0. Results are mean ± standard deviation of three experiments and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures.

Table 1
Quantities of L- and D-amino acids (mmol/l) and relative amounts of isomers (%*L* and %*D*) resulting from the Maillard reaction

		Glc/Gly	Glc/L-Asn	Glc/D-Asn	Glc/L-Lys	Glc/D-Lys	Fru/Gly	Fru/L-Asn	Fru/D-Asn	Fru/L-Lys	Fru/D-Lys
pH 4.0	L-AAs	65.71 ± 3.29[B]	110.99 ± 5.55[Aa]	11.85 ± 0.59[b]	100.87 ± 5.04[a]	7.02 ± 0.35[c]	123.73 ± 6.19	178.84 ± 8.94[a]	14.18 ± 0.71[b]	165.40 ± 8.27[a]	13.08 ± 0.65[b]
	D-AAs		11.90 ± 0.59[d]	115.94 ± 5.80[b]	53.53 ± 2.68[c]	257.02 ± 12.85[a]		17.23 ± 0.86[c]	190.03 ± 9.50[a]	37.69 ± 1.88[b]	192.53 ± 9.63[a]
	%*L*	–	–	9.27 ± 0.46	–	2.66 ± 0.13	–	–	6.95 ± 0.35	–	6.36 ± 0.32
	%*D*	–	9.68 ± 0.48	–	34.67 ± 1.73	–		8.79 ± 0.44	–	18.56 ± 0.93	–
pH 7.0	L-AAs	126.71 ± 6.34	128.63 ± 6.43[a]	11.38 ± 0.57[c]	119.57 ± 5.98[a]	30.90 ± 1.54[b]	190.54 ± 9.53	187.58 ± 9.38[a]	24.59 ± 1.23[c]	125.66 ± 6.28[b]	16.83 ± 0.84[d]
	D-AAs		9.32 ± 0.47[d]	160.44 ± 8.02[b]	42.15 ± 2.11[c]	183.32 ± 9.16[a]		6.65 ± 0.33[d]	186.12 ± 9.31[a]	30.40 ± 1.52[c]	132.06 ± 6.60[b]
	%*L*	–	–	6.62 ± 0.33	–	14.43 ± 0.72	–	–	11.67 ± 0.58	–	11.31 ± 0.57
	%*D*		6.75 ± 0.34	–	26.06 ± 1.30	–		3.42 ± 0.17	–	19.48 ± 0.97	–
pH 10.0	L-AAs	709.56 ± 10.64	596.35 ± 8.95[a]	26.64 ± 0.53[d]	196.68 ± 3.93[b]	40.20 ± 0.80[c]	370.08 ± 7.40	62.56 ± 1.25[b]	54.71 ± 1.09[c]	123.06 ± 2.46[a]	19.98 ± 0.40[d]
	D-AAs		31.34 ± 0.63[d]	589.89 ± 8.85[a]	126.91 ± 2.54[c]	266.98 ± 5.34[b]		49.60 ± 0.99[d]	535.82 ± 8.04[a]	62.98 ± 1.26[c]	116.51 ± 2.33[b]
	%*L*	–	–	4.32 ± 0.22	–	13.09 ± 0.65	–	–	9.27 ± 0.46	–	14.64 ± 0.73
	%*D*		4.99 ± 0.25	–	39.22 ± 1.96	–		44.22 ± 2.21	–	33.85 ± 1.69	–

[a–d]Means in a column followed by different superscripts are significantly different at the $p < 0.05$ level.
[A] Values are means of three replicates ± standard deviation.
[B] Remaining achiral glycine.

3.3. Browning and formation of melanoidins

The browning development of melanoidins before and after dialysis is shown in Fig. 2. The final stage of the browning reaction was monitored by the increase in absorbance at 420 nm (Ajandouz et al., 2001). In the present study, browning development was increased as increasing pH levels. Browning development of fructose-amino acid systems was higher than glucose-amino acid systems. Browning development was showed depending on the pH levels, being more significant for model systems apart from heated glucose solution alone. On the other hand, as L- and D-isomer of amino acid used, browning development is not shown different characteristics, except for Glc/L-Asn and Glc/D-Asn model system at pH 4.0. The majority of colored compounds after dialysis were not retained in the high-molecular-weight fraction (Mr > 3500) but below it. This result is in line with literature describing browning in sugar–amino acid systems. Leong (1999) observed that the high-molecular-weight fraction (>3500 Da) contributed only up to 10% of the absorbance of the glucose/glycine reaction mixture heated in acetate buffer at 55 °C and pH 5.5. Also Hofmann (1998) in both glucose/glycine and glucose/alanine systems heated in phosphate buffer for 4 h at 95 °C, pH 7, reported that only trace amounts of compounds with molecular weights greater than 3000 Da were formed. These results are in contrast with sugar/protein reactions. A much higher percentage of color was detected in the high-molecular-weight fraction ($\geqslant 70\%$), which is as expected since the melanoidins are attached to the protein that is high-molecular-weight by itself (Brands, Wedzicha, & Van Boekel, 2002; Hofmann, 1998). Color in MRPs is almost exclusively due to the low-molecular-weight fraction.

The wavelength spectra of melanoidins before and after dialysis are shown in Figs. 3 and 4, where absorbance is increased as increasing pH levels and after dialysis for all model systems. The L- and D-isomers both show different absorption in the UV–vis spectra and that these occur at similar shape. Every peak has a stable absorbance appeared in the range between 260 nm and 320 nm, characteristic of melanoidins. This trend was also described by other authors for melanoidins-type colorants (Guimaraes, Bento, & Mota, 1996; Rafik, Mas, Elharfi, & Schue, 1997). Before dialysis, at pH 4.0 and 7.0, the band intensities for the two isomers are different: the low absorption curve is formed for the L-isomer whereas the higher absorption curve is formed for the D-isomer in asparagine model systems. However, the low absorption curve is formed for the D-isomer whereas the higher absorption curve is formed for the L-isomer in lysine model systems. The highest absorption curve is formed for the L-isomer in lysine model system. The higher absorption curve was formed in glucose-amino acid systems than fructose-amino acid systems. On the other hand, at pH 10.0, Fru/L-Lys and Fru/D-Lys were formed for the low absorption curve than Glc/L-Lys and Glc/D-Lys. After dialysis, the absorption was higher than those of before dialysis. The highest absorption curve

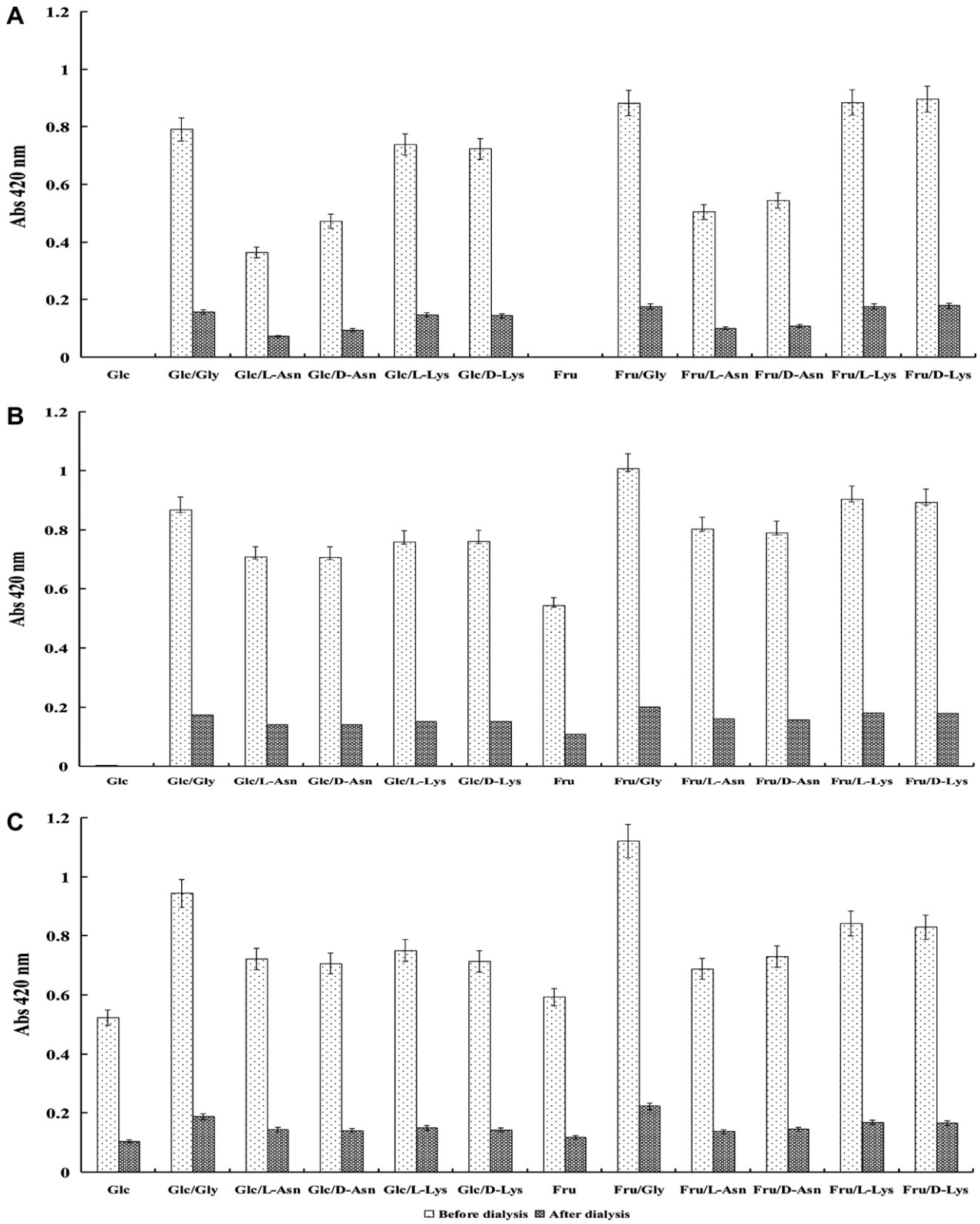

Fig. 2. Development of browning (as measured by absorbance at 420 nm) of melanoidins before and after dialysis in glucose and fructose/L- or D-amino acid model system. (A) pH 4.0; (B) pH 7.0 and (C) pH 10.0. Results are mean ± standard deviation of three experiments and data are evaluated by using one-way analysis of variance was performed by ANOVA procedures.

is formed in Fru/Gly. After crossing the UV region the absorption curve progressively came into the blue-absorbing region of the visible spectrum and yellow, orange, brown colors appeared. The compounds formed early in the Maillard reaction absorb in the UV, e.g. HMF, but do not absorb in the visible region of the spectrum.

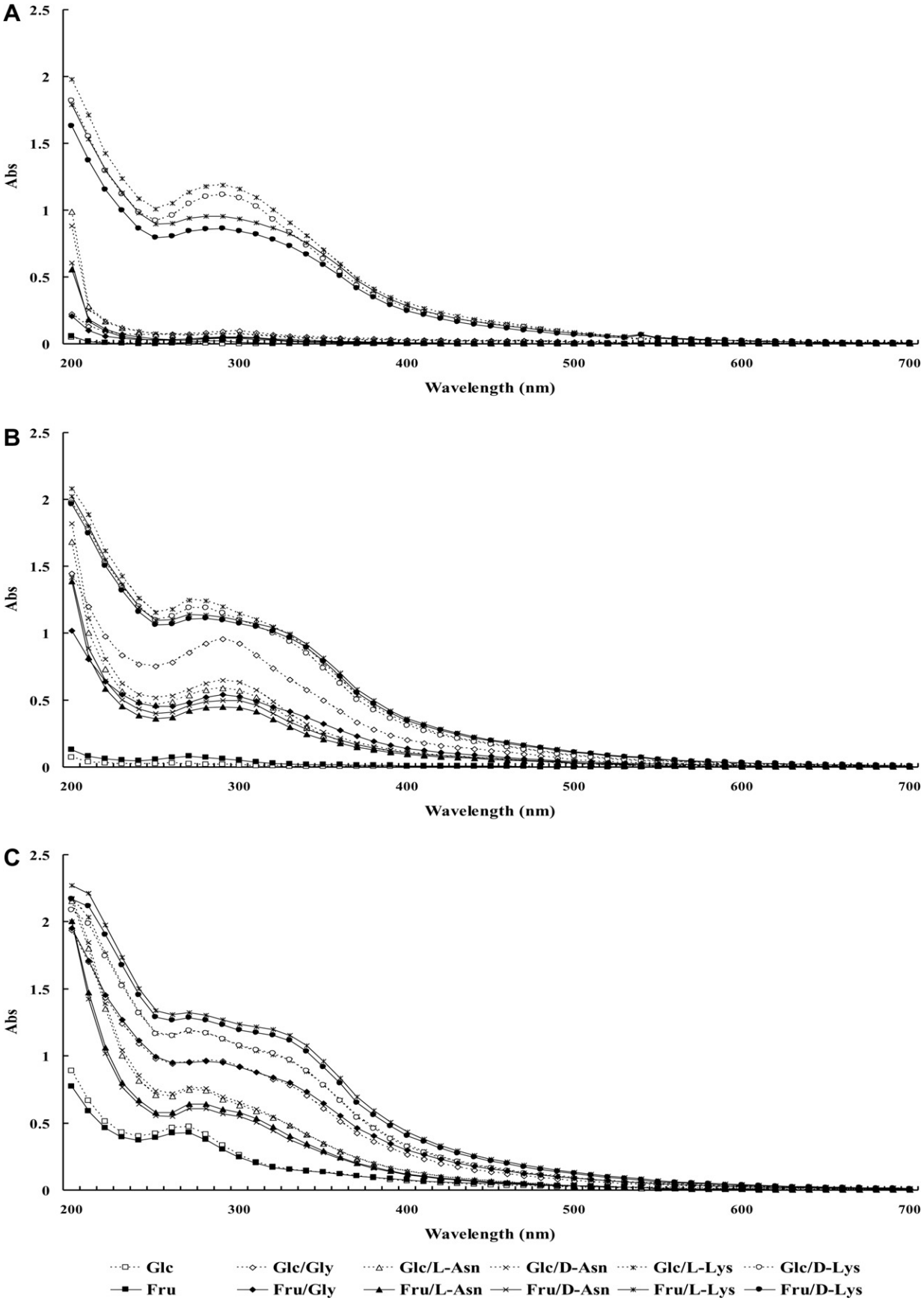

Fig. 3. Comparison of the UV–vis spectra of melanoidins before dialysis in glucose and fructose/L- or D-amino acid model system. (A) pH 4.0; (B) pH 7.0 and (C) pH 10.0.

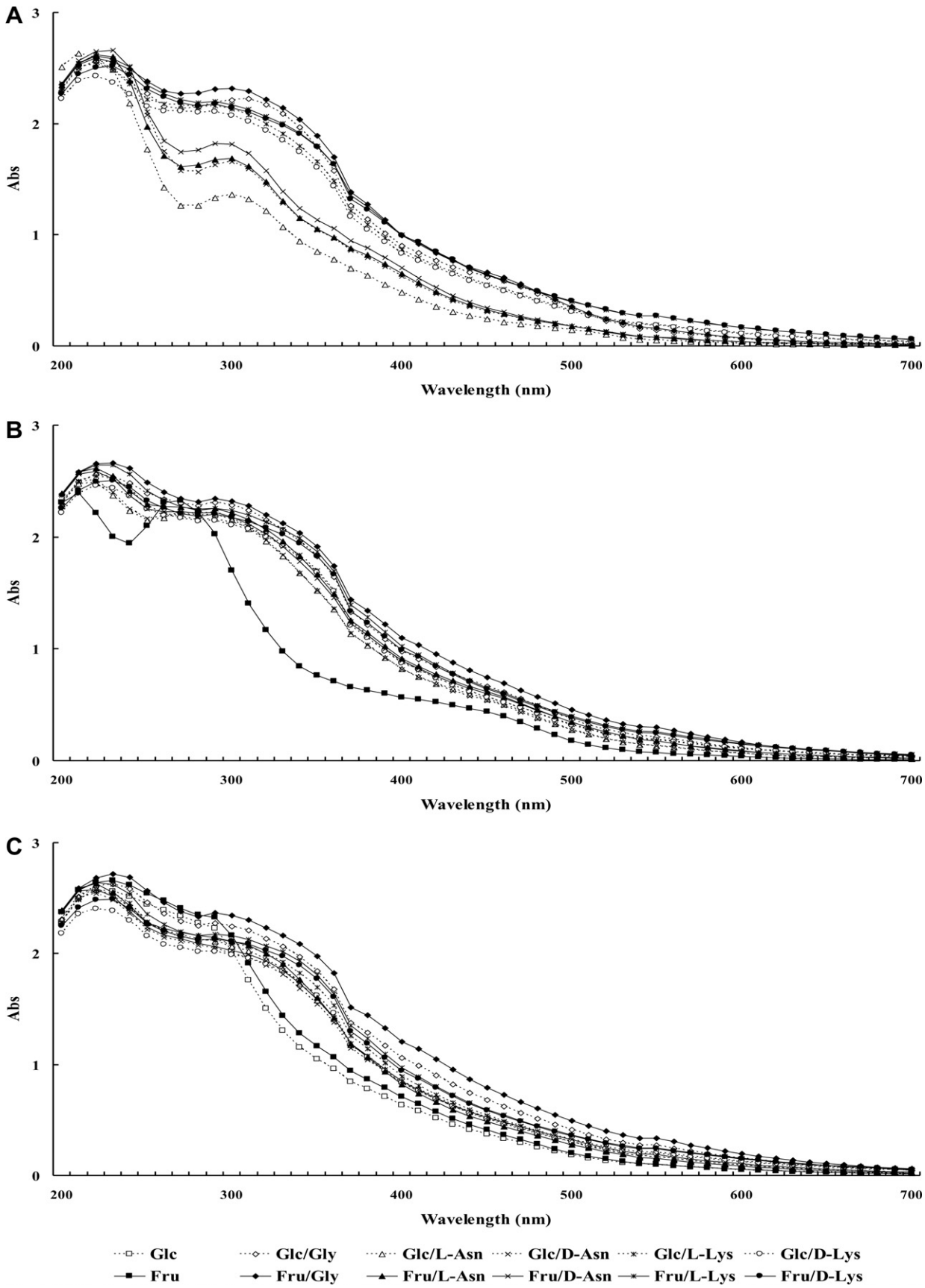

Fig. 4. Comparison of the UV–vis spectra of melanoidins after dialysis in glucose and fructose/L- or D-amino acid model system. (A) pH 4.0; (B) pH 7.0 and (C) pH 10.0.

4. Conclusion

This study was to investigate the effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers, can influence the formation of melanoidins as result of the Maillard reaction. The result of this study proves that D (L)-amino acids are formed on heating aqueous solutions of L (D)-amino acids and saccharides. Melanoidins is formed by D-amino acids are similar to those by the L-amino acid. Presence of amino acids together with saccharides is common. Thus, the study provides also a feasible explanation for the generation of free D-amino acids in foods or biological systems which is not depending on microorganisms or racemases (Brückner & Schieber, 2000; Erbe & Brückner, 2000). Furthermore, it is important to evaluate for color development and melanoidins extinction coefficient in the course of the Maillard reaction. Color of melanoidins formed in model systems might be related to the enolization of sugars and racemization of amino acids. Therefore, it could be more useful to the understanding of the Maillard reaction and it might be extended on the reaction of amino acids with reactive carbonyl compounds in general.

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