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RESEARCH NOTE

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FT-IR and X-Ray Diffraction Characterization of Melanoidins Formed from Glucose and Fructose with Amino Acid Enantiomers in the Maillard Reaction

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Abstract The objective of this study was to investigate Fourier transform infrared (FT-IR) spectrometry and the X-ray diffraction (XRD) characterization of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction. Before dialysis, FT-IR spectroscopy of all the samples showed that the characteristic absorption intensities appeared as a broad and intense band of the stretching vibration of the -OH group at 3,400/cm for a high pH. The absorption bands of the melanoidins sharply decreased in intensity after dialysis as compared to those before dialysis. In particular, the absorption bands at 992 and 575/cm disappeared. The XRD confirmed that the crystal structure of the melanoidins disappeared after dialysis and a new crystal structure was formed at 9 and 28° (2θ). In particular, broad diffraction peaks were formed in the 10-21° (2θ) range for a high pH, while other sharp diffraction peaks disappeared.

Keywords: amino acid enantiomer, Maillard reaction, melanoidin, Fourier transform infrared (FT-IR) spectrometry, X-ray diffraction

Introduction

Melanoidins are dark brown to black colored natural condensation products of sugars and amino acids produced by non-enzymatic browning reactions called Maillard reactions (1). Naturally melanoidins are widely distributed in food (2) and drinks and widely discharged in huge amount by various agro-based industries especially from cane molasses based distilleries and fermentation industries as environmental pollutants (3). The structure of melanoidins is still not completely understood but it is assumed that it does not have a definite structure as its elemental composition (4) and chemical structures largely depend on the nature and molar concentration of parent reacting compounds and reaction conditions as pH, temperature, heating time, and solvent system used (5).

Although the chemical structure of melanoidins is not understood clearly, some part of the chemical structure of model melanoidins have recently been elucidated by different spectral studies such as ^{1}H-nuclear magnetic resonance (NMR), ^{15}N cross polarized-magnetic angle spinning (CP-MAS) NMR, etc (4,6,7). The chemical investigations have revealed that natural and synthetic melanoidins both have similar elemental (CHON) compositions, spectroscopic properties, and electrophoretic mobilities at various pH values (4,6). However, nitrogen contents, acidities, and electrophoretic behavior of the polymers all reflect functional group distributions inherited from the amino acids (8). Benzing *et al*. (9) have studied xylose-glycine (N^{15}) melanoidin by CP-MAS NMR and reported that the nitrogen in melanoidin polymers exists mainly in secondary amide form and some as pyrrole and/or indole nitrogen and they also revealed that sterically hindered secondary amide bonds are very resistant to acid hydrolysis. According to Hayase *et al*. (10) the melanoidins structure seems to have CH_3-COR moiety and C-terminal structures originated from glucose existing in melanoidins. However, Cammerer *et al*. (11) have proposed the basic structure of melanoidins formed from 3-deoxyhexosuloses and Amadori reaction products. In spite of these studies, the melanoidins chromophore has not been yet identified. Hence, the chemical structure of the so-called melanoidin is still not clear but probably it does not have a definite one and there exists various types of melanoidins differing in structure depending on parent reactants and reaction conditions as pH, temperature, and reaction time. Moreover, it further needs intensive investigations with more refined recent and advanced techniques for the elucidation of chromophore structure to deduce the main skeleton of melanoidin polymer.

The Maillard reaction can also explain the formation of D-amino acids in food. Brückner *et al*. (12) 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 hr in aqueous solutions of pH 2.5 (AcOH) or 7.0 (NaOAc). Thus, the formation of D-amino acids in many foods of plant and animal origin are the results of nonenzymic browning since the presence of amino acids together with saccharides is common. Recently, heating experiments of synthetic Amadori compounds proved that they are sources of amino acid enantiomers (13-15). Furthermore, convincing evidence has been recently established that D-amino acids are formed in the course of the Maillard reaction (12-14).

Therefore, the objective of this study was to investigate the Fourier transform infrared (FT-IR) spectrometry and the X-ray diffraction (XRD) characterization of melanoidins

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formed from glucose and fructose with amino acid enantiomers in the Maillard reaction. Melanoidins were, rather arbitrarily, defined as being a high molecular weight (HMW) with a lower limit of 3.5 kDa, which was the nominal cut-off value in the dialysis system used.

Materials and Methods

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

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. Eight model systems were prepared, being glucose/L-asparagine (Glu/L-Asn), glucose/D-asparagine (Glu/D-Asn), glucose/L-lysine (Glu/L-Lys), glucose/D-lysine (Glu/D-Lys), 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

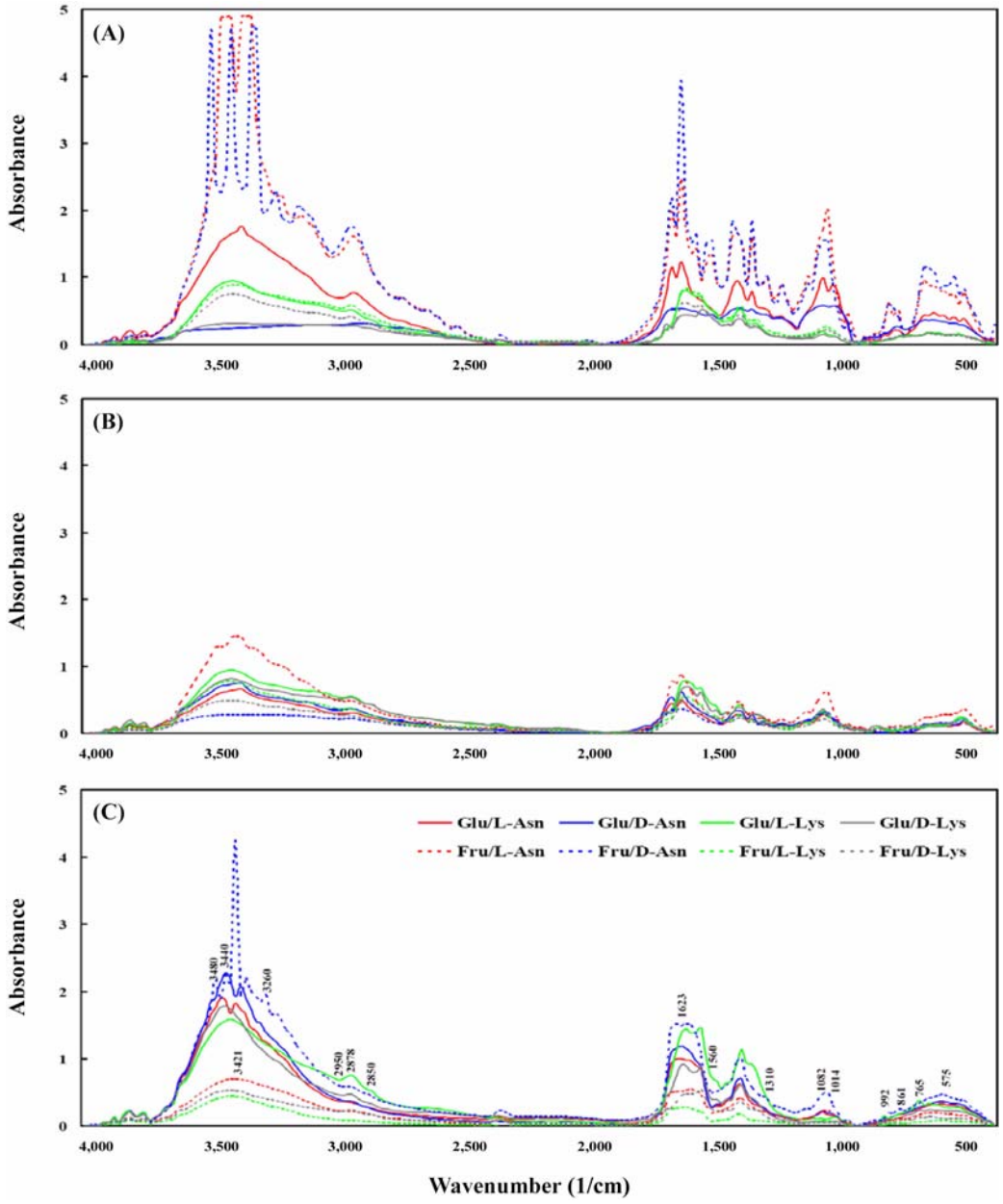

Fig. 1. FT-IR spectra of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction before dialysis. A, pH 4.0; B, pH 7.0; C, pH 10.0.

mm), each containing a minimum of 10 mL. Model solutions were heated at 100°C for 2 hr without pH control in at least duplicate. The heating was carried out in a silicone oil bath and the proper safety measures are taken. After heating, model solutions were immediately cooled in ice water and then dialysed or undialysed.

Dialysis Approximately 2 mL of the reaction mixture were injected into Slide-Alyzer dialysis cassettes (Mw> 3,500) (3.5K MWCO; Pierce, Rockford, IL, USA) and dialyzed against distilled water. A batch dialysis was performed against 1,500 mL of double distilled water for 168 hr at 4°C. Water was changed every 3 hr for the first 12 hr, and then every 10-12 hr for the rest of the dialysis time. After dialysis, samples were freeze-dried and stored in a dessicator at 4°C until analysis. The MRP samples before and after dialysis were dissolved in water before use.

FT-IR analysis The FT-IR analysis was performed using an IFS 28CS FT-IR spectrometer (Bruker, Rheinstetten, Karlsruhe, Germany). The samples were collected using the KBr pellet method. FT-IR spectra were recorded at a resolution of 4/cm and with a total of 100 scans, and wave number range between 400 and 4,000/cm.

XRD analysis The XRD was obtained from a DMAX-IIIA X-ray diffractometer (Rigaku, Tokyo, Japan), a conventional copper target X-ray tube set to 40 kV and 30 mA. The X-ray source was Cu Kα radiation. Data were collected from 2θ of 3.00 to 50.00° (θ being the angle of diffraction) with a step width of 0.02° and step time of 0.4 sec, scanning speed of 5°/min, divergence slit width of 0.2

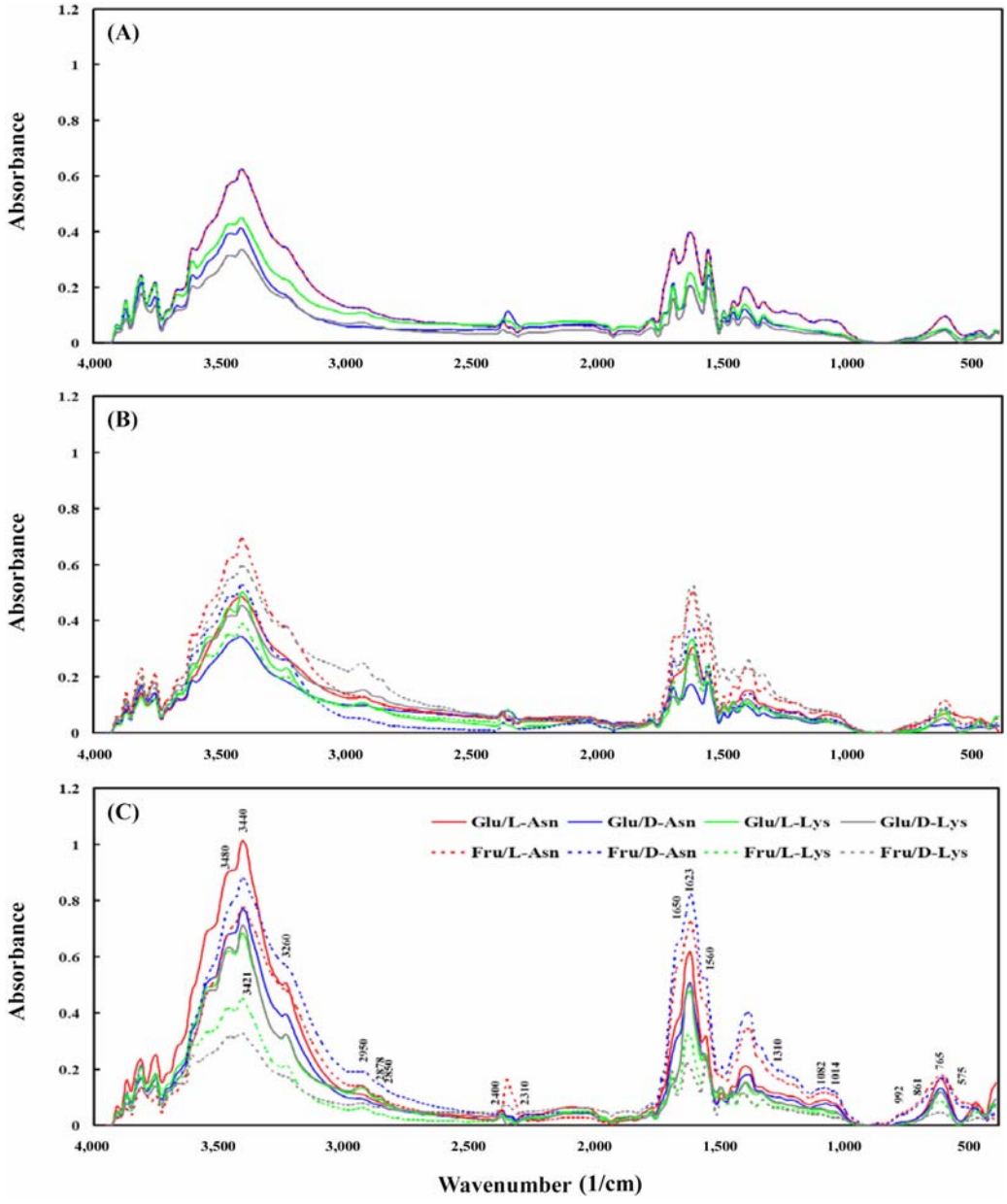

Fig. 2. FT-IR spectra of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction after dialysis. A, pH 4.0; B, pH 7.0; C, pH 10.0.

mm, scatter slit width of 0.6 mm, receiving slit width of 0.2 mm at room temperature. Samples were freeze-dried, and then 50 mg samples were added into the slide for packing prior to X-ray scanning.

Results and Discussion

FT-IR spectra The FT-IR spectrum of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction is shown in Fig. 1 and 2. It is similar to previously reported spectra for other synthetic melanoidins (16). Before dialysis, as increasing pH, all the samples showed the broad and intense band of the stretching vibration of the -OH group at 3,400/cm. An extremely broad band due to hydrogen bonded hydroxyl groups appeared at 3,421/cm (17). The main bands were due to oxygen-containing functions such as those at 3,440/cm (OH groups) and at 1,706/cm (COOH groups). The relatively high intensity of these bands was consistent with the fact that the melanoidins were prepared with an excess of sugars. The low contribution of the CH_2 and CH_3 groups was reflected by the weak absorption in the 2,850-2,950/cm range. The characteristic absorption bands between 4,000 and 2,500/cm were 3,480-3,440, 3,260-3,270, and 2,960-2,878/cm for the OH, NH, and the CH stretching regions, respectively (18). Additional interesting information on the chemical composition of the melanoidins was obtained from observations of the bands at 1,623 and 1,510/cm; these bands are usually attributed to aromatic furanic and conjugated compounds. Additional characteristic

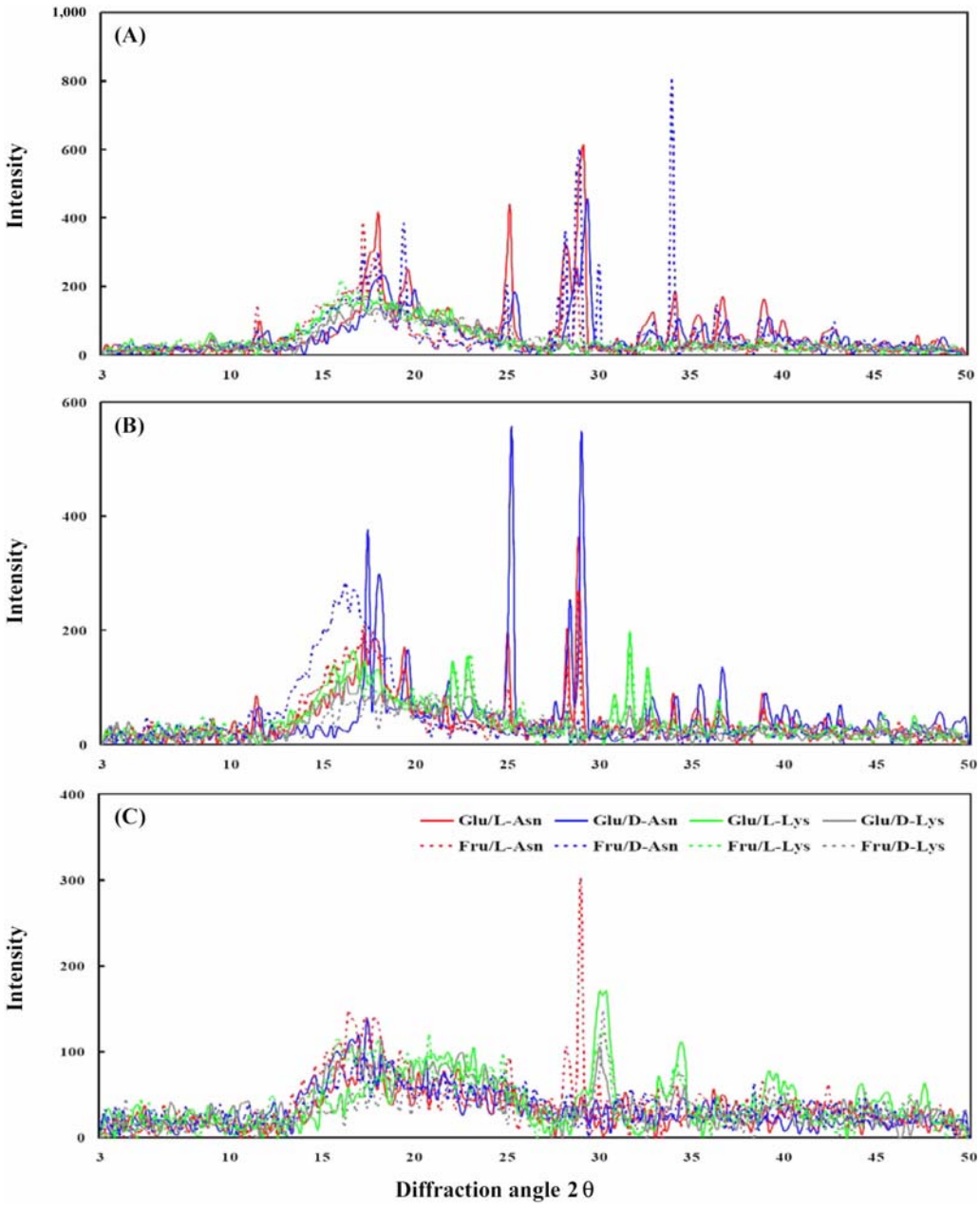

Fig. 3. X-ray diffraction pattern of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction before dialysis. A, pH 4.0; B, pH 7.0; C, pH 10.0.

absorption bands appeared at 992, 929, 861, 765, and 575/cm due to the stretching vibrations of the entire anhydroglucose ring. The adsorption bands around 1,650, 1,560, and 1,310/cm were attributed to amide I, II, and III groups (19), respectively. In addition, there were several discernible absorbancies at 1,159, 1,082, and 1,014/cm, which were attributed to C-O bond stretching (20). After dialysis, the absorption bands weakened significantly. In particular, the absorption band in the range 2,310-2,400/cm was newly formed. Therefore, this suggested that the melanoidins contained OH, NH, CH_2, CH_3, amide I, II, and III groups. In addition, the chemical composition of the melanoidins was also obtained from the bands of aromatic furanic and conjugated compounds. Slight differences were observed between the bands around 992 and 575 and in the range 2,310-2,400/cm before and after dialysis. This could be due to the difference in the reactivities, which depend on the molecular weight and chemical structure.

XRD analysis XRD measurements were performed to examine if chemical modifications altered the crystallinity of the melanoidins. The XRD spectra of the melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction are shown in Fig. 3 and 4. Before dialysis, as increasing pH, all the samples showed sharp diffraction peaks. In particular, broad

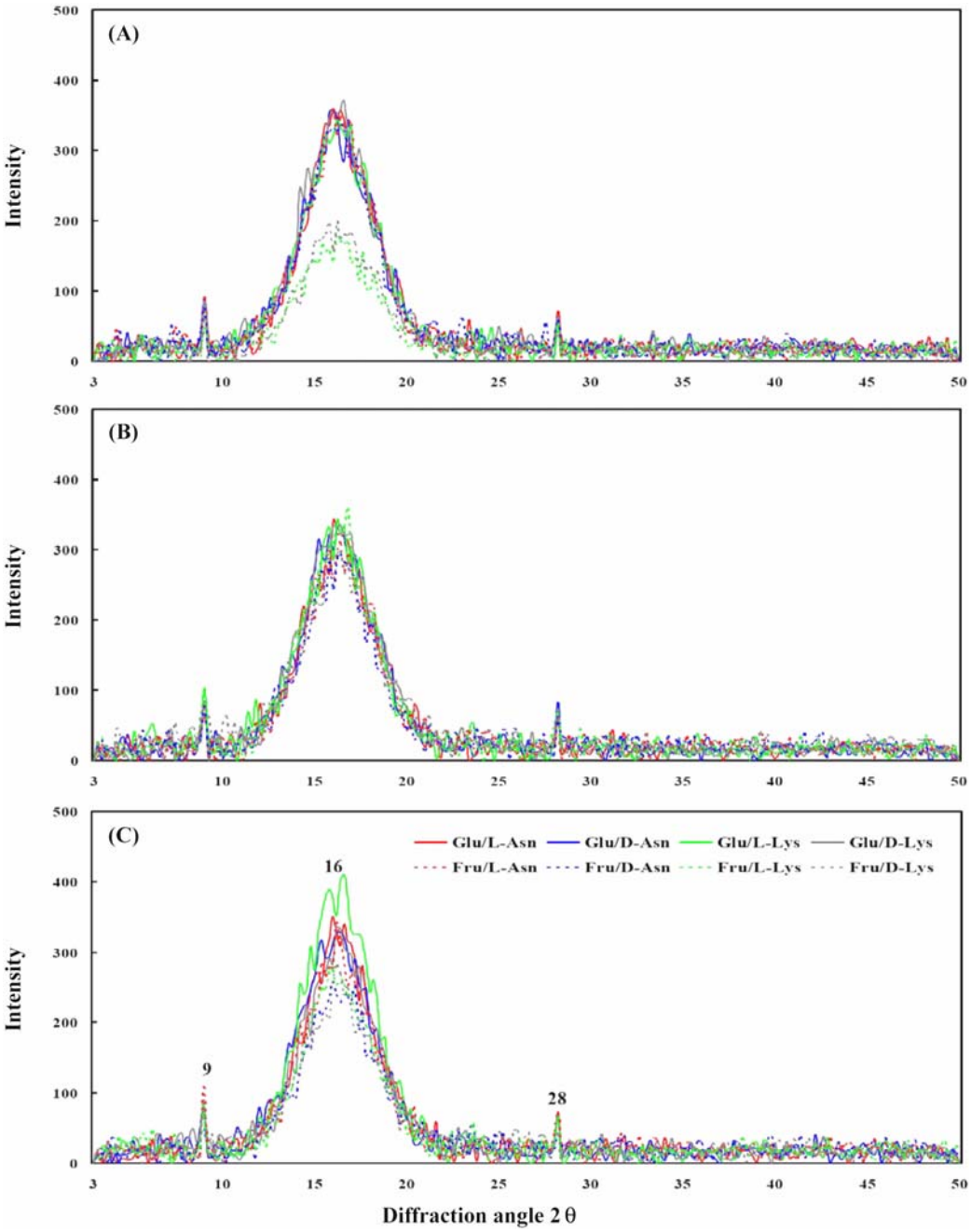

Fig. 4. X-ray diffraction pattern of melanoidins formed from glucose and fructose with amino acid enantiomers in the Maillard reaction after dialysis. A, pH 4.0; B, pH 7.0; C, pH 10.0.

diffraction peaks were formed in the 10-21° (2θ) range as increasing pH, while other sharp diffraction peaks disappeared. After dialysis, all the samples showed similar profiles of the broad diffraction peaks in the 10-21° (2θ) range; they also showed a new peak at 9 and 28° (2θ). However, the intensity of the diffraction peaks was lower than their intensities in other systems such as Fru/L-Lys and Fru/D-Lys systems at pH 4. This observation suggested that the crystallinity of the melanoidins was formed in the 10-21° (2θ) range. As the pH increased, the crystallinity of the melanoidins also increased. In addition, the crystallinity of the melanoidins formed from L-isomers was similar to the crystallinities of D-isomers, but had a different magnitude.

In conclusion, the melanoidins studied contained OH, NH, CH_2, CH_3, and amide I, II, and III groups. In particular, melanoidins formed from glucose-based systems had a stronger structure than those formed from fructose-based systems. The chemical composition of melanoidins was obtained from the absorption bands of aromatic furanic and conjugated compounds. The crystallinity of the melanoidins products was formed in the 10-21° (2θ) range. As the pH increased, the crystallinity of the melanoidins also increased. The crystallinity of melanoidins obtained from L- or D-isomers was similar to the crystallinity of the isomers; however, it varied in magnitude. Therefore, these results suggested that the melanoidins have different FT-IR spectra pattern according to type of sugars and pH level. Especially, the crystallinity of melanoidins is formed by D-amino acids are similar to those by the L-amino acid.

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