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Application of capillary zone electrophoresis to the study of food and food-model melanoidins

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Abstract

Different water soluble melanoidins have been isolated from both heated (100 °C/24 h) carbohydrate/amino acid model systems and medium-roasted coffee. Capillary zone electrophoresis (50 mM sodium tetraborate, pH 9.3) has been applied to determine the saturated or aromatic character of the isolated melanoidins. Melanoidins obtained from different solutions after an equivalent heating treatment possess similar apparent molecular weight but different charge/mass ratio, suggesting differences in their degree of saturation. Melanoidins isolated from model systems containing lysine showed a lower saturation than those isolated from either coffee or other model systems containing glycine, alanine or tryptophan. Depending on the type of amino acid as reactant, melanoidins could be mainly constituted by a stable structure or common core which is changing according to the thermal conditions applied through a more saturated structure. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Maillard reaction; Melanoidin; Capillary zone electrophoresis; Gel filtration

1. Introduction

Melanoidins are polymeric and coloured final products of the Maillard reaction (MR), where reducing carbohydrates or their degradation products react with compounds possessing a free amino group. MR depends upon factors such as pH, time, temperature, moisture content, and type and concentration of reactants (Ames, 1992). Melanoidins are responsible for the colour in cooked and processed foods, and they are present in widely consumed dietary components (e.g. coffee, bread, and roasted malt). During recent years, interest in these compounds has increased because of their nutritional (antiradical, antimutagenic, and chelating properties; Lee & Kwon, 1998) and physical (viscosity, and colour; Hofmann, 1998a) characteristics. Due to this growing interest in the effect of melanoidins of the human diet and their feasible nutritional, biological and health implications, the European Union has launched a specific COST (Co-operation in Science and Technology) action entitled "Melanoidins in Food and Health" (COST, 1998).

Many research efforts have been done to determine the structure and chemical properties of melanoidins but since none has been isolated and characterised yet, this information is still lacking. An approximation on its structure has been gained through infrared (IR) spectroscopy, mass spectrometry and advanced multidimensional nuclear magnetic resonance spectroscopy (NMR) analyses (Arnoldi, Corian, Scaglioni, & Amis, 1997; Hofmann, 1998b; Milic, 1987). Cämmerer and Kroh (1995) suggested a melanoidin structure based on the reactions (aldol reaction or nucleophilic addition) of dicarbonyl compounds with themselves (mainly, 3-deosyosones), as well a substitution reaction with amino compounds, intramolecular cyclisation, decarboxylations and water elimination. Therefore, it cannot be assumed that the melanoidins have a regular composition with repeating units. Analytical separation of melanoidins has been attempted through gel or paper electrophoretic techniques according to the belief of an amphoretic nature (Hashiba, 1978; O'Reilly, 1983). However, later studies by using capillary zone electrophoresis (CZE) have shown that these compounds possess a partially anionic character in solution over a wide pH range (Tomlinson & Landers, 1993). On the other hand, reversed-phase HPLC of melanoidins is considered unsatisfactory, since they are chemically ill-

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defined and consist of a complex mixture of compounds (Royle, Bailey, & Ames, 1998).

Nowadays, CZE has been proved to be a powerful and very promising technique for food analysis, because of its high efficiency and resolving power, small sample and buffers requirements. This paper deals with the application of CZE on the separation and analysis of different melanoidins isolated from model systems and coffee.

2. Materials and methods

2.1. Chemicals

All chemicals used were the purest available. D-(+)-Glucose, L-tryptophan, L-lysine monohydrochloride, L-alanine, and L-glycine monohydrochloride were purchased from Sigma (St. Louis, MO, USA), and D-(+)-lactose monohydrate from Panreac (Barcelona, Spain).

2.2. Preparation of water-soluble melanoidins

2.2.1. From aqueous Maillard reaction (MR) model systems

Sugar (0.1 mol) and amino acid (0.1 mol) were dissolved in 1 l of a 100 mM phosphate buffer (pH 7) solution. Eight model systems were prepared: glucose–alanine (GA), glucose–glycine (GG), glucose–lysine (GL), glucose–tryptophan (GT), lactose–alanine (LA), lactose–glycine (LG), lactose–lysine (LL), and lactose–tryptophan (LT). Model solutions were heated without pH control in tightly stoppered Pyrex flasks immersed in a glycerol bath at 100 °C for 24 h. In a second experiment, GG and GL systems were heated up to 120 hours, and samples were taken at 6, 24, 48 and 120 h of treatment. In both experiments, a portion of the MR mixtures (10 ml) was added to a wet cellulose dialysis tubing (33 mm of flat width, 12.4 kDa of MWCO, Sigma). Batch dialysis was performed against 1.5 l of double distilled water during 136 h. Water was changed every 3 h the first 12 h and then every 10–12 h. After dialysis, samples were freeze-dried and stored in a desiccator at 4 °C. Before analysis, a standard melanoidin solution (3 mg/ml) was prepared in water. After centrifugation (8000*g* for 5 min at 4°C) and filtration (0.45 μm) to remove possible insoluble material, sample was ready for both HPGPC and CZE analysis.

2.2.2. From roasted coffee

Medium roasted coffee powder was purchased from a local store. Two different procedures were carried out. In procedure (a) coffee brews were prepared with ground coffee (12 g) and 100 ml of distilled water using a domestic coffee machine. This coffee solution, named as CD, was filtered though a Whatman No. 4 paper filter and a 10 aliquot dialysed and lyophilised as described in Section 2.2.1. Procedure (b) was done following the recommendation of COST-919 group for coffee-melanoidin analysis (COST, 1998). Ground coffee (100 g) was stirred in 300 ml of distilled water at 75 °C for 5 min. This solution, named as CH, was filtered and an aliquot of filtrate was de-fatted with dichloromethane (2×200 ml). This coffee solution was then dialysed and lyophilised as described in Section 2.2.1.

2.3. High-performance gel-permeation chromatography (HPGPC) analysis

A TSK-GEL 3000SW column (60 cm×7.5 mm ID, TosoHaas, Germany) was used. The injection volume was 10 μL for model solutions and 20 μL for melanoidin standards (3 mg/ml), and detection at 280 nm and 420 nm was selected. Samples were eluted in double-distilled water at 0.8 ml/min. A Kontron Instruments (Milan, Italy) chromatographic system was used. Void column volume was calculated with a standard solution of 1 mg/ml blue dextran (2000 kDa) diluted in 50 mM sodium phosphate at pH 7.

2.4. Capillary zone electrophoresis (CZE) analysis

Electromigration was carried out with a HP^{3D} system equipped with a built-in diode-array detector and a HP ChemStation for system control, data collection, and data analysis from Hewlett-Packard (Madrid, Spain). CZE was performed on an uncoated fused silica capillary with 48.5 cm total length (40 cm effective length), 50 μm internal diameter, and an extended light path (bubble factor of 3) supplied by Hewlett-Packard (Madrid, Spain). Capillary was conditioned by washing with 1 M NaOH for 20 min, wait for 5 min, water for 5 min, and then buffer for 20 min. Samples were hydrodynamically injected by applying 50 mbar pressure for 2 s at the anionic end of the capillary. After every run capillary was hiflushed (7 bar) with 0.1 M NaOH (0.2 min), and 50 mM sodium tetraborate pH 9.3 for 0.4 min. Electrode buffers were renewed every five runs with 0.5 ml per pot. Temperature of the cartridge was maintained at 25 °C, and total separation time was set at 15 min. A standard electrophoretic run was performed at a constant voltage of 25 kV with the anode at the inlet side, reaching a current of about 96 μA. Electropherograms were monitored at 214, 280, and 420 nm wavelength, with raw spectral data collection between 190 and 500 nm. Benzyl alcohol was used as marker of the electroosmotic flow (EOF) and a benzoic acid solution (1 mg/ml) as internal standard.

2.5. Measurement of browning

Browning indices of the different MR mixtures were recorded after appropriate dilution by their absorbance

at 420 nm on a Shimadzu UV-1601 (Duisburg, Germany) spectrophotometer using a 1-cm path length cell.

3. Results and discussion

The extent of the MR in the different mixtures and coffee solutions was monitored by measurement of the absorbance at 420 nm (Table 1). The systems containing lysine and tryptophan reached higher browning values at the experimental heating condition. The CH coffee solution obtained by the second procedure (recommended by the COST-919 group) gave a browning level approximately 2.7 times higher than that of CD coffee solution (procedure a). However, no great difference was observed in the recovery of melanoidins from coffee between both solutions. Similar levels of hydroxymethylfurfural, a heat-induced marker for thermally processed foods, were also found in both coffee systems (5.59 and 6.14 μmol/g of coffee for CD and CH coffee brews, respectively). On the other hand, absorbance at 420 nm of the heated MR mixtures was highly related to the total melanoidin recovery ($r^2 = 0.824$) when heating conditions were standardised at 100 °C for 24 h, independently of the carbohydrate or amino acid used, (Table 1). The MR systems containing tryptophan did not follow the same trend, giving slightly higher rates of recovery at the same browning measurements. No precipitation in the isolated melanoidin fraction was observed when it was reconstituted in water. During heating of MR mixtures containing tryptophan, a fine precipitate was produced but this was removed by filtration. Fig. 1 shows the linear relationship between browning and melanoidin recovery for GG and GL model systems heated up to 120 h at 100 °C. It appears that an induction period is necessary to reach the final stage of the MR where melanoidins are formed as final products. Since a minimal absorbance value of 0.041 is required for melanoidin formation. Melanoidin recovery was nearly 6 times higher for the coffee brews than MR mixtures. This observation could be due to the presence of other compounds, such as polyphenol structures, which are involved in the formation of melanoidins with phenolic subunits in coffee solutions (Heinrich & Baltes, 1987).

Table 1
Browning (absorbance at 420 nm) and recovery of melanoidins (mg/100 mL) from different Maillard reaction mixtures and coffee solutions[a]

Sample	A_{420}	Recovery
GG	0.255	74.1
LG	0.233	88.6
GT	0.557	180.0
LT	0.573	196.9
GL	0.484	125.0
LL	0.334	73.3
GA	0.199	70.6
LA	0.161	83.5
CD	0.528	607.5
CH	1.418	871.3

[a] CD, coffee solution obtained by procedure a; CH, coffee solution obtained by procedure b. Average of duplicate measurements.

Fig. 2 depicts the complexity of some MR mixtures and coffee. To evaluate the efficiency of the dialysis procedure, the melanoidin fraction of each model system was analysed by HPGPC. Additionally, absence of glucose or lactose in the retained fraction was confirmed by ion-exchange HPLC analysis. Regardless of the type of sugar used, systems containing glycine, alanine or lysine showed a well-defined peak of high molecular weight melanoidin (Fig. 2a). Whereas in the system containing tryptophan, isolated melanoidin was eluted in two peaks (Fig. 2b). These results show that melanoidins obtained from different sources under the same heating conditions possess similar apparent molecular weight according to their retention times in gel-filtration analysis. Coffee melanoidins were also eluted as a single peak. All the melanoidins were eluted at about 11.8 min. and the void volume was set at 8.5 min (dextran blue).

CZE was applied to study the complexity of the MR mixtures, and to give some insight on the ionic character of the melanoidins isolated from different model systems. Analytical conditions were adjusted using the previous studies of Tomlinson and Landers (1993) and Royle et al. (1998). Fig. 3a shows the electropherograms of LG model system and coffee. Each MR mixture had several predominant peaks at 214 and 280 nm with the visible fraction (420 nm) always correlated with the hump in the electropherogram. Hill et al. (1998) applied CZE to separate a glycine–lysine mixture where many well-resolved peaks were superimposed on a broad band of coloured material of high molecular weight. Fig. 3b depicts the electropherograms of some isolated mela-

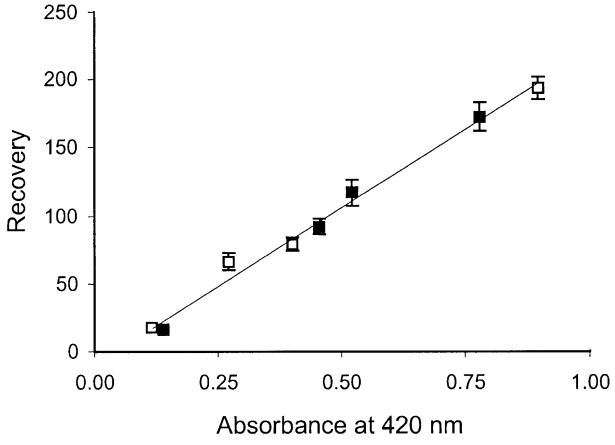

Fig. 1. Correlation between browning (determined by the absorbance at 420 nm) and melanoidin recovery (mg/100 mL) in two model systems heated up to 120 h. ($r^2 = 0.9899$). GG (□), GL (■). Error bars show standard deviation.

noidins. Again, the type of carbohydrate was not relevant in the complexity of the electropherograms, the type of amino acid used being the most important feature for the ionic properties of melanoidins. The hump observed in each MR mixture corresponds with the migration time of the isolated melanoidins. HPGPC analysis gives a rather similar apparent molecular weight but CZE analysis shows a different charge/mass ratio. For instance, the electrophoretic pattern was similar for systems containing glycine and alanine, and different from that obtained from systems containing lysine. During CZE analysis, neutral compounds migrate with the EOF, while cations and anions have lower and higher migration times, respectively. The electrophoretic migration velocity of the solute ion is largely governed by its size and number of ionic charges. Therefore, a more charged melanoidin will move faster than a less charged melanoidin charges and similar size. For MR products, Royle et al. (1998) stated that negative charge could be either because they are an anionic behaviour at pH 9.3 or because their form an anionic complex with borate. Then, it could be assumed then that borate complexation also plays a role in the anionic migration character of melanoidins. On the other hand,

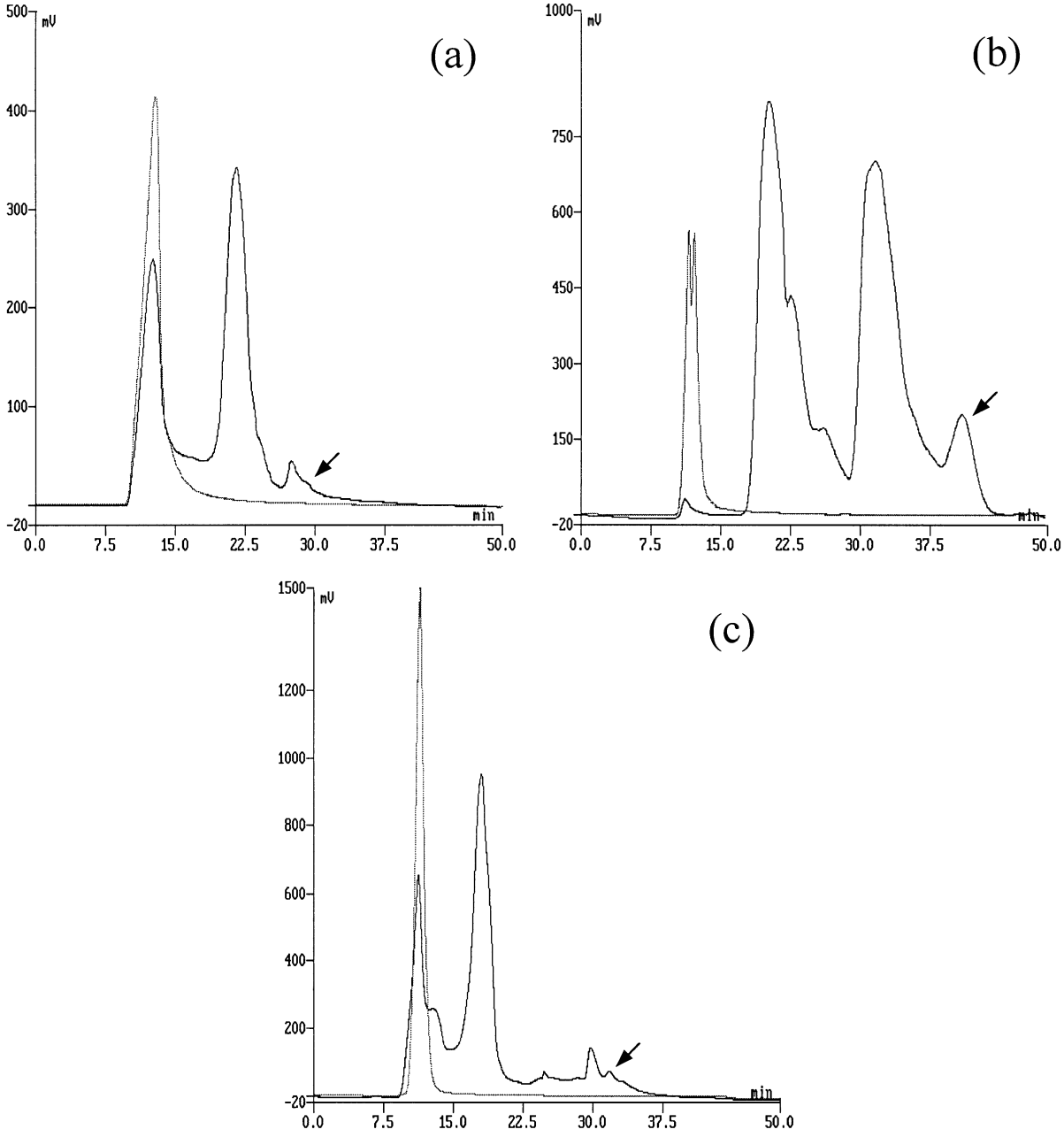

Fig. 2. Elution patterns of MR mixtures (arrow) and their corresponding non-dialyzable melanoidins by HPGPC analysis. Detection was at 420 nm; (a) LG system, (b) LT system, and (c) coffee.

the velocity of EOF is affected by the degree of ionisation of the silanol groups of the capillary, since at pH above 8 all the silanol groups will be charged, and the EOF migration time will be maximum. Under the conditions used in this study, the EOF was about 2.52 min as determined by using benzylic alcohol. Electrophoretic conditions were tested daily with a benzoic acid solution showing a migration time of about 6.64 min.

The net electrophoretic velocity (cm/s) was calculated from the ionic mobility of the melanoidin at the applied electric field (given by V/L_t, where V is the applied voltage in volts and L_t is the total length in cm of the capillary tube). Negative values were obtained due to the anionic character of melanoidins at these pH conditions. Net electrophoretic velocities of −160.3, −170.1, −148.9, −143.6, −114.8, −121.9, 161.1 and −169.3 cm/s were obtained for LG, GG, LT, GT, LL, GL, LA, and GA mixtures, respectively. Differences observed due to the procedure of preparation of coffee melanoidins were with values of −125.9 and −124.9 cm/s for the CD and CH systems, respectively. As a reference, benzoic acid solution gave a net electrophoretic velocity of −164.1 cm/s.

These results show that melanoidins formed from different MR mixtures should have different reactive residues, but similar apparent molecular weight at the same heating conditions and molar ratio of reactants. Benzing-Purdie et al. (1985) described in xylose/glucose and glycine systems that with increasing reaction time and temperature, the total carbon content increases at the cost of the nitrogen ratio, thus promoting the aromatic character and the unsaturation of melanoidins. Additionally, infrared and ^{13}C-NMR analyses of melanoidins showed an increase in aromaticity with reaction temperature. In this study, reaction conditions were constant and it was found that melanoidins obtained form different sources have different anionic character related to their degree of unsaturation. Melanoidins isolated from model systems containing lysine had less anionic behaviour at pH 9.3 than that obtained from the other systems and from coffee. Both coffee–melanoidin had a hump at the left side of the peak (Fig. 3b, 4). These could show the complexity of melanoidin compound formed, where a single structure is not generated during roasting of coffee beans.

An additional experiment was designed to investigate how the charge/mass properties of melanoidins were affected by the experimental conditions. Fig. 4 shows the electropherograms of GG and GL model systems treated at 100 °C for up 120 h. Melanoidins obtained from GL mixtures showed a relatively constant shape of the electrophoretic peak in all samples taken along the heating period. An increment of the non-water-soluble melanoidin fraction was observed in samples heated for longer than 24 h. On the other hand, melanoidins obtained from GG mixtures slightly changed their electrophoretic patterns and had a reduced net electrophoretic mobility. From these results it could be feasible to propose that a main melanoidin structure is formed at low heating conditions in the lysine-containing model system and is stable over the heating time (Fig. 4a). On the other hand, GG-melanoidin structure undergoes some evolution, and GG-melanoidin increases its saturated or aromatic character with longer heating times

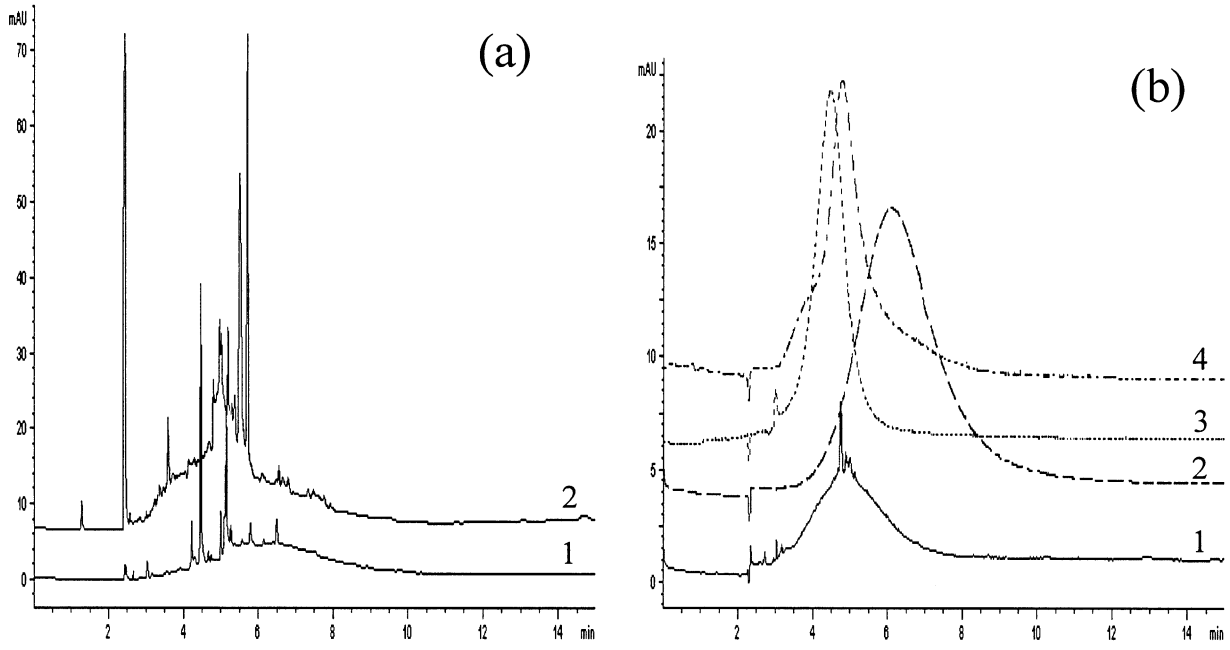

Fig. 3. Electropherograms of the LG reaction mixture (1a) and coffee (2a) recorded at 280 nm (a), and of LT (1b), LG (2b) and LL (3b) melanoidin standard solutions and coffee (4b), recorded at 420 nm (b).

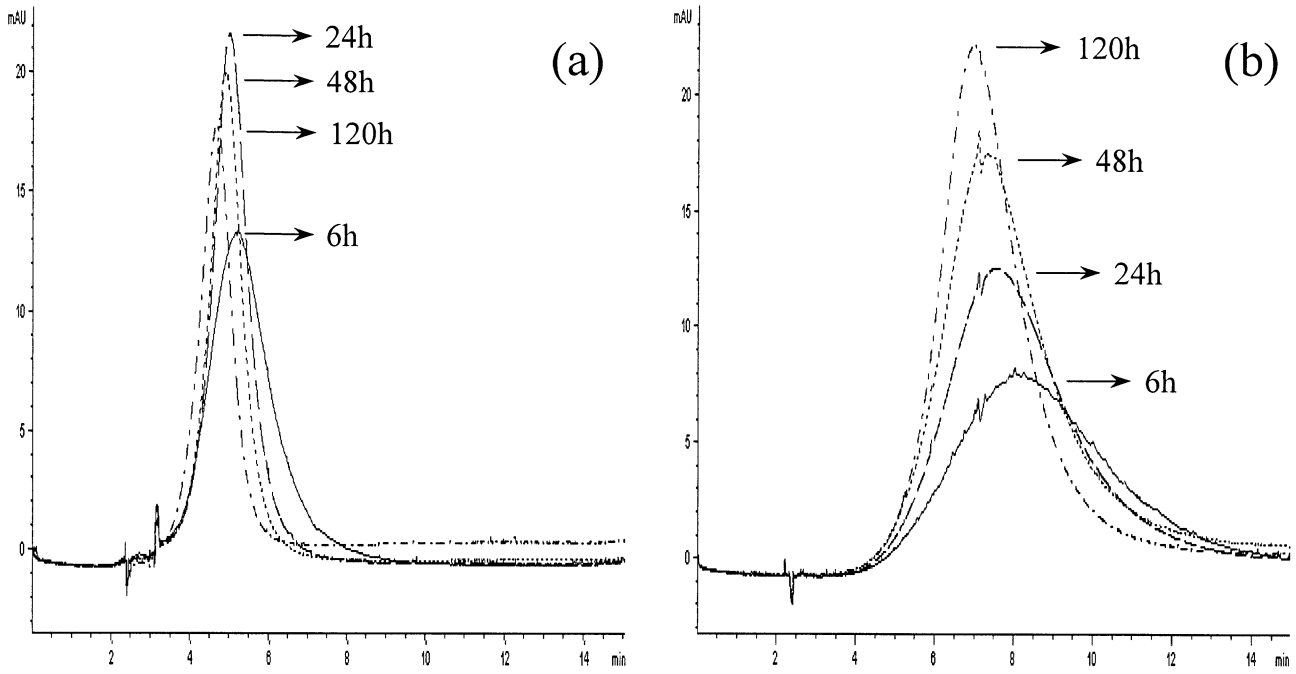

Fig. 4. Electropherograms of melanoidins obtained from GL and GG mixtures, respectively, heated at 100 °C for up to 120 h.

(Fig. 4b). Broadness is related to an undefined group of compounds with similar structures. The broadness of the GG-melanoidin peak is reduced with increasing heating times indicating that a well-defined structure is being formed. In contrast, a main structure is formed in the GL-melanoidin independently of the heating conditions. Structural differences in the melanoidins obtained from different sources are confirmed. These results could help to understand some functional properties of melanoidins, such as their antioxidant capacities in foods, flavour binding properties or their biological activity in humans.

4. Conclusions

The hump observed in the CZE analysis of the MR mixtures agrees with the migration time of the respective isolated melanoidins, showing a clear anionic character at pH 9.3. Melanoidins obtained from different reagents at standardised heating conditions possess similar apparent molecular weights but different charge/mass ratios indicating a different degree of unsaturation. For instance, melanoidins isolated from lysine-containing systems showed a more unsaturated behaviour than isolated from a system containing glycine, alanine, or tryptophan and from coffee. On the other hand, depending on the type of amino acid, melanoidin is constituted by a common structure (i.e. in the system containing lysine) or changes towards a more saturated one when heating temperature increases (i.e. in the system containing glycine).

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