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Flavor Release in the Presence of Melanoidins Prepared from L-(+)-Ascorbic Acid and Amino Acids

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High-molecular-weight (HMW) water-soluble melanoidins were prepared from model systems of L-(+)-ascorbic acid−glycine, L-(+)-ascorbic acid−lysine, L-(+)-ascorbic acid−glutamic acid, and glucose−glycine using a very recently approved standard protocol. The amount of HMW water-soluble melanoidins prepared from L-(+)-ascorbic acid was over 5−15 times higher than the amount obtained from glucose. The study of the release of a model flavor compound, namely isoamyl acetate, from melanoidins by solid-phase microextraction (SPME) showed that SPME is a suitable technique for the analysis of flavor release from melanoidin-containing solutions. From the studies on the retention capacity of the melanoidins toward isoamyl acetate, an increased release of the flavor compound was observed from the melanoidins prepared from the L-(+)-ascorbic acid−glycine model system, whereas the opposite effect was observed from the melanoidins prepared from the L-(+)-ascorbic acid−lysine/glutamic acid model systems. The melanoidins prepared from the glucose−glycine model system had the same effect as the melanoidins prepared from the L-(+)-ascorbic acid−glycine model system.

KEYWORDS: Flavor release; melanoidins; isoamyl acetate; 2-phenylethanol; vitamin C; amino acids; retention capacity

INTRODUCTION

Flavor is one of the basic indicators of the quality of food products. The behavior of the individual aroma components is determined by their volatility and their interaction with the compounds of the nutrition product. These complex physicochemical processes have been studied in model systems containing β-lactoglobulin, ovalbumin, and other proteins (*1*−*4*).

Apart from biopolymers, the food products obtained by thermal treatment also contain chemical heteropolymers, called melanoidins. They are a result of the Maillard reaction, an interaction between reducing carbohydrates, furan derivatives, etc., with free amino acids or proteins (*5*).

Participation of L-(+)-ascorbic acid in the Maillard reaction as a carbonyl compound is one of the newest directions in the studies of its numerous manifestations. The interaction of L-(+)-ascorbic acid with ammonia and different amino acids and its effect on the nonenzymatic browning of the reaction products have been studied by measuring the absorbance of the reaction mixture (*6*−*12*).

There are only a few reports on melanoidins derived from the interaction of L-(+)-ascorbic acid and nitrogen compounds (*12*−*15*). In a series of studies Rogacheva et al. (*16*−*19*) and Vernin et al. (*20*) investigated the mechanisms and kinetics of the generation of melanoidins from glycine, lysine, and glutamic acid.

The nonenzymatic browning of juices and some food concentrates is partly due to L-(+)-ascorbic acid. Its participation and turnover during the Maillard reaction leads to the accumulation of organic acids, furan derivatives, ketones, cyclopentanones, pyranones, or pyrroles that could change their flavor (*21*−*24*).

The process of melanoidin formation has been studied in a model of apple juice with and without adding L-(+)-ascorbic acid (*25*). The authors have determined the reaction constant and activating energy of the reaction. The melanoidins obtained this way, and the products of their thermal degradation, were isolated and characterized.

Hofmann et al. (*26*) suggested that the melanoidins in coffee, being polyfunctional polymers, could covalently bind to aroma compounds. They added the total melanoidin fraction isolated by water extraction from medium-roasted coffee powder to a model solution containing a set of 25 aroma compounds, mimicking the aroma of a coffee brew. Reduction of the intensity of the roasty and sulfury aroma quality was observed.

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Table 1. Synthetic Data and Reaction Yields of Melanoidins Prepared from Various Model Systems

model system	carbonyl compound (g)	amino acid (g)	total (g)	raw reaction product (g)	water-insoluble melanoidins (g)	HMW water-soluble melanoidins (g)
Glc–Gly	9.00	3.70	12.70	8.65	5.00	0.06
vit C–Gly	8.91	3.80	12.71	10.56	1.14	0.60
vit C–Lys	8.86	7.30	16.17	14.11	0	0.91
vit C–Glu	8.75	7.37	16.12	14.99	0	0.30

Table 2. UV-Absorption of Solution A (5 g of Reaction Mixture in 250 mL of Water)

	absorption at wavelength					λ_{max} (nm)	
model system	280 nm	360 nm	420 nm	460 nm	520 nm	before dialysis	after dialysis
Glc–Gly	0.640	0.261	0.058	0.030	0.013	294	270
vit C–Gly	1.086	0.084	0.034	0.020	0.011	280	271
vit C–Lys	0.876	0.085	0.031	0.019	0.009	280	290
vit C–Glu	0.538	0.037	0.013	0.007	0.001	265	270

The low-molecular-weight melanoidins (1500−3000 Da) led to a most significant decrease in 2-furfurylthiol. The aldehydes remained unaffected by melanoidins. In a very recent study by the same group (*27*), the chemical interactions between thiols responsible for coffee aroma and melanoidins were investigated. In the presence of melanoidins a drastic decrease in the concentration of some odorous thiols, especially 2-furfurylthiol, in the headspace of fresh coffee brews was observed.

The content of L-(+)-ascorbic acid-derived melanoidins in numerous food products makes the study of their effect on the behavior of the aroma complex necessary. In the current study the effect of melanoidins prepared from L-(+)-ascorbic acid and three different amino acids on the behavior of certain aroma components in model conditions is shown.

This study on the flavor release from melanoidins is related to the current COST 919 (Cooperation in Science and Technology) action on melanoidins, and this is one of the first times that these types of flavor release studies on standard and related melanoidins have been carried out. As model flavor compounds, an ester (namely isoamyl acetate) and an aromatic alcohol (2-phenylethanol) were selected because they are slightly soluble in water and are widely used as flavor and fragrance materials.

MATERIALS AND METHODS

Materials. Glycine (>99%) and D-glucose (99%) were obtained from Sigma (Bornem, Belgium). L-(+)-Ascorbic acid (>99%), L-(+)-lysine hydrate (99%), L-glutamic acid (99%), and the model flavor compounds isoamyl acetate (99%) and 2-phenylethanol (99%) were purchased from Acros Organics (Geel, Belgium). Dialysis tubing with a flat width of 33 mm was purchased from Sigma. This cellulose membrane retains >90% cytochrome *c* (MW 12 400) in solution over a 10-h period. The dialysis tubing was prepared according to the manufacturer's instructions. UV spectra were recorded with a Cary 50 UV spectrophotometer (Varian) with a data interval of 1 nm and a fixed slit of 1.5 nm.

Preparation of Melanoidins. 0.05 Mol of glucose (9.00 g) or L-(+)-ascorbic acid (8.80 g) and 0.05 mol of glycine (3.75 g), lysine (7.30 g), or glutamic acid (7.35 g) were placed in a 300-mL Christ filter bottle and dissolved in 20 mL (80 mL for glutamic acid) of distilled water. The solution was frozen in a bath of liquid nitrogen. Subsequently, it was freeze-dried (Christ alpha 1−4) until all the water had been removed (i.e., up to constant weight). The carbonyl compound−amino acid mixture was placed in an oven (Memmert) which was equipped with a fan and had been preheated to and stabilized at 125 °C. The mixture was heated for exactly 2 h without covering. After heating, the filter bottle was allowed to cool to room temperature in a desiccator. The solid was transferred to a mortar and carefully ground to a fine powder. A 5-g aliquot of the ground material was added to 200 mL of distilled water, and the solution was stirred for 12 h at 4 °C to dissolve as much material as possible. This suspension was filtered through Whatman no. 4 filter paper, and the filtrate (which contained the water-soluble melanoidins) was collected. The residue on the filter paper was washed with 2 × 20 mL of distilled water, and the liquid obtained after washing was mixed with the original filtrate. This solution (solution A) was made up to 250 mL with distilled water in a volumetric flask. The residue obtained, the so-called water-insoluble fraction of the melanoidins, was frozen, freeze-dried, and stored at −32 °C until further use. The UV−visible absorbance spectrum (200−600 nm) of solution A was obtained as follows: 1 mL of solution A was diluted to 100 mL with distilled water and the absorbances at 280, 360, 420, 460, and 520 nm were recorded in particular.

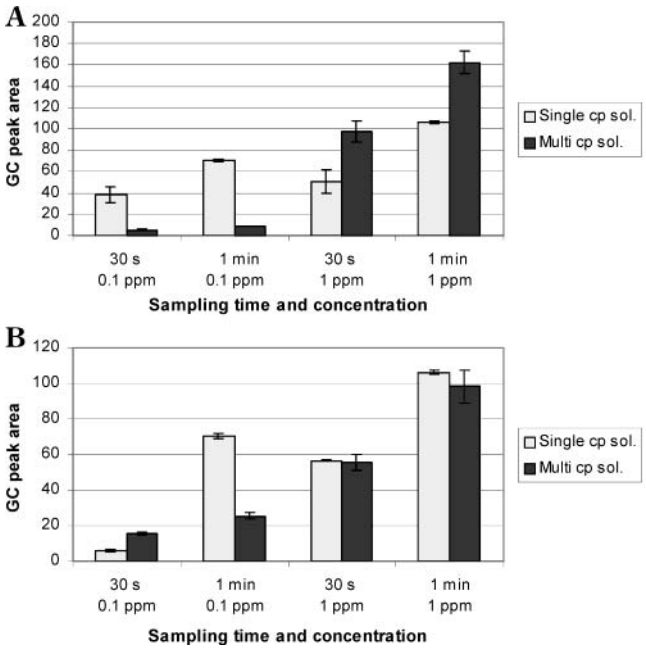

Figure 1. Extraction of isoamyl acetate from a single-compound solution and a multi-compound solution containing 2-phenylethanol: A, test in aqueous solutions without melanoidins; B, test in solutions containing 100 ppm model melanoidins (glucose–glycine melanoidins).

Isolation of Nondialyzable Melanoidins by Dialysis. Solution A (50 mL) was brought in 21 cm of dialysis tubing and was dialyzed against 1 L of distilled water for 24 h at 4 °C with two changes of the surrounding water. The dialysates, containing the low-molecular-weight (LMW) fraction of melanoidins were not collected. At the end of the dialysis the contents of the dialysis tubing with the high-molecular-weight fraction (HMW), or so-called nondialyzable melanoidins, were transferred to a 500-mL round-bottom flask, frozen in a liquid nitrogen bath, and freeze-dried until all the water had been removed. The HMW

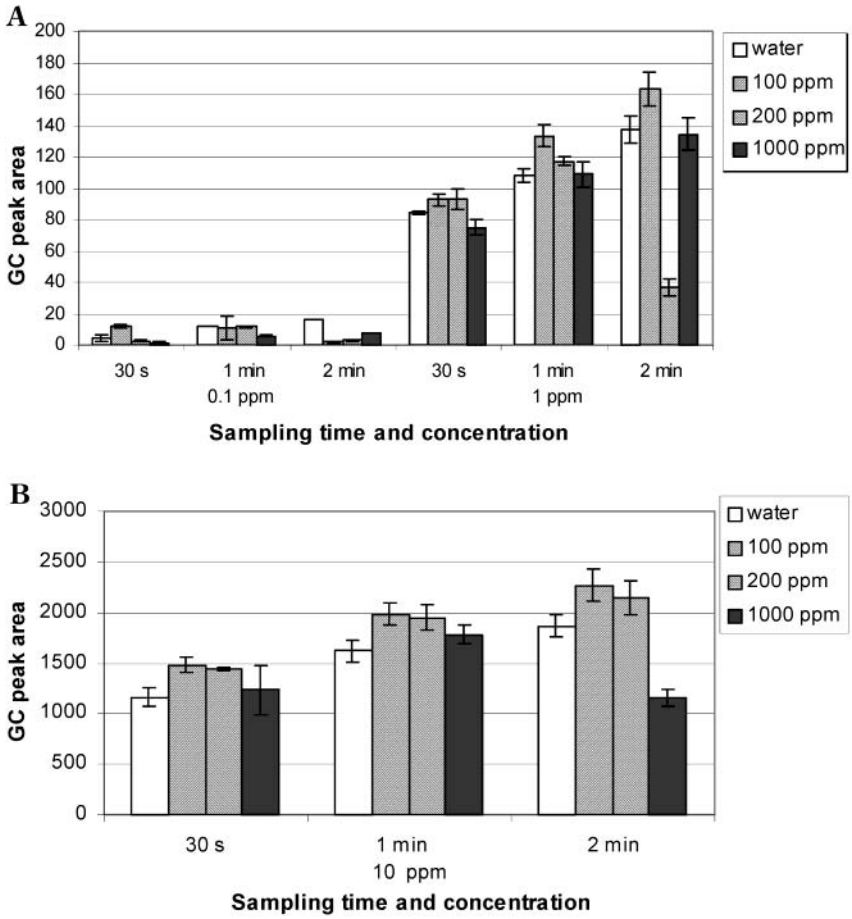

Figure 2. Isoamyl acetate retention capacity of melanoidins prepared from a glucose–Gly mixture (test in water and in aqueous solutions with melanoidin concentrations of 100, 200, and 1000 ppm): A, low (0.1 ppm) and moderate (1 ppm) concentration of isoamyl acetate; B, high concentration (10 ppm) of isoamyl acetate.

water-soluble melanoidins were stored in a desiccator in a freezer at −32 °C until further use.

Flavor-Release Studies. To study the release of the model flavor compound, isoamyl acetate, and the effect of the other flavor compound, 2-phenylethanol, on the release of isoamyl acetate, solutions of the different model melanoidins in water at different concentrations were prepared, namely 100, 200, and 1000 ppm. In all cases melanoidins were freshly prepared and were of the same age. In general, the HMW melanoidins were ready for use after 4 days, i.e., on the fifth day after the initial mixing of the amino acid and the sugar. The model flavor compounds were dissolved in these melanoidin-containing solutions at concentrations of 0.1, 1, and 10 ppm, either alone (single-compound solution) or together (multi-compound solution). The release of the model flavor compound from the melanoidin-containing solutions into the headspace was measured by means of solid-phase micro-extraction (SPME) as the sampling technique, because this method offers a simple and sensitive technique for volatile compound analysis and it samples primarily the headspace (*28*). Therefore, 1 mL of a solution with melanoidins and the model flavor compound was pipetted into a 4-mL silanized vial (Supelco Inc., Bellefonte, PA) and stirred (800 rpm). After 30 min of equilibration time, an SPME extract was taken from the headspace using a PDMS (100-μm) fiber (Supelco) during 30 s, 1 min, or 2 min at 25 °C.

Analysis of the SPME Extracts. After SPME extraction, the SPME fiber was desorbed during 1 min at 250 °C in the chromatographic inlet of the GC. GC analyses were performed with a HP 6890 GC Plus apparatus, equipped with a split/splitless injector, an FID detector, and an EC-5 column (30 m length × 0.25 mm i.d.; coating thickness 0.25 μm). Operating conditions were as follows: injector 250 °C; detector 300 °C (makeup gas He at 10 mL/min); oven temperature, start 60 °C (1 min), programmed from 60 to 120 °C at 10 °C/min, from 120 to 160 °C at 20 °C/min, hold 2 min; carrier gas (He) 21.4 cm/s; splitless injection.

RESULTS AND DISCUSSION

HMW water-soluble melanoidins were obtained from the model systems glucose/L-(+)-ascorbic acid−glycine, lysine, and glutamic acid in a molar ratio of 1:1 in anhydrous medium at 125 °C (Materials and Methods, Preparation of Model Systems) (**Table 1**). During their synthesis the reaction mixtures lost about 7 to 31.9% of their initial weight. In the glucose−glycine model system the loss was significantly higher (31.9%) than in the systems in which the carbonyl compound was L-(+)-ascorbic acid (16.9%). In the L-(+)-ascorbic acid−glutamic acid model system the loss was only 7%.

HMW water-insoluble melanoidins were obtained only in the case of the glycine−glucose and the glycine−L-(+)-ascorbic acid model systems (5.0 and 1.14 g, respectively). In these two cases the polymerization reaction, i.e., Maillard reaction, is faster compared to the analogous model reactions with lysine and glutamic acid.

The yield of HMW water-soluble melanoidins obtained by using L-(+)-ascorbic acid was 5 to 15 times higher (0.3−0.91 g) than the same melanoidin fraction obtained by using glucose (0.06 g) (**Table 1**). These results demonstrate that the melanoidin synthesis is strongly affected by the chemical nature of the carbonyl compound and the amino component in the Maillard reaction.

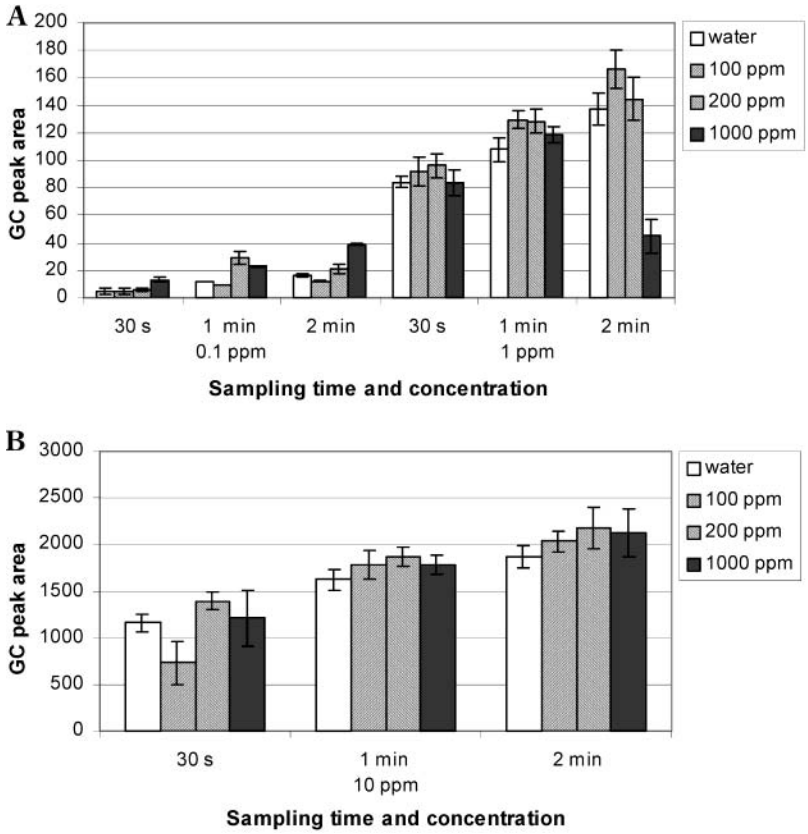

Figure 3. Isoamyl acetate retention capacity of melanoidins prepared from a vitamin C–Gly mixture (test in water and in aqueous solutions with melanoidin concentrations of 100, 200, and 1000 ppm): A, low (0.1 ppm) and moderate (1 ppm) concentration of isoamyl acetate; B, high concentration (10 ppm) of isoamyl acetate.

The reaction mixtures of the L-(+)-ascorbic acid−glycine and L-(+)-ascorbic acid−lysine model systems showed higher absorption at 280 nm than the mixture of the glucose−glycine model system (**Table 2**). In contrast, at all the other wavelengths the glucose−glycine model system demonstrated a higher absorption. The reaction mixture of the L-(+)-ascorbic acid−glutamic acid model system had the lowest absorption at all the studied wavelengths. In addition, the λ_{max} of the reaction mixture before dialysis and the λ_{max} of the nondialyzable melanoidins differed significantly (5−24 nm), indicating that the presence and the concentration of chromophores is different before and after dialysis.

The flavor release was studied by solid-phase micro-extraction (SPME). In a first experiment isoamyl acetate was chosen as the model flavor compound and the effect of 2-phenylethanol on its release was studied in a multi-compound solution. Two extraction times (30 s and 1 min) and two different concentrations of aroma compounds (0.1 and 1 ppm) were compared in this study. In an aqueous solution without melanoidins and in the presence of 2-phenylethanol (**Figure 1A**) the amount of the extracted isoamyl acetate decreased when a low concentration of flavor compounds was applied (0.1 ppm), but increased when a higher concentration was used (1 ppm). Therefore, it can be concluded that at higher concentration (1 ppm) 2-phenylethanol behaves mainly as a solvating agent. In a system containing 100 ppm of model melanoidins (prepared from glucose and glycine), the effect of 2-phenylethanol on the release of isoamyl acetate was different than that in the aqueous solution without melanoidins (**Figure 1B**). At a lower concentration of the flavor compounds (0.1 ppm), the effect was dependent on the sampling time. A significant increase in isoamyl acetate release (double) was observed after 30 s sampling, whereas a strong decrease was noticed after 1 min. The changes in release obtained at higher concentration (1 ppm) were not significant.

In a next set of experiments, the release of the model flavor compound isoamyl acetate from melanoidin-containing solutions was investigated by SPME and compared with the release from aqueous solutions. Three extraction times (30 s, 1 min, and 2 min), three different concentrations of model flavor compound (0.1, 1, and 10 ppm), as well as three different melanoidin concentrations (100, 200, and 1000 ppm) were compared in this study. Moreover, four different melanoidins were investigated, namely the model melanoidin obtained from the glucose−glycine mixture and three types of melanoidins prepared with vitamin C, namely as a mixture with glycine, with lysine, and with glutamic acid.

The isoamyl acetate retention capacity of melanoidins prepared from the glucose−glycine mixture was rather different depending on the experimental conditions (**Figure 2**). At the lowest concentration of isoamyl acetate (0.1 ppm) the presence of melanoidins decreased the release of the aroma compound. The low concentration of the model flavor compound probably favors its more complete binding to the heterogeneous melanoidin backbone. At higher concentrations (1 and 10 ppm) the peak areas of isoamyl acetate increased, i.e., the release of isoamyl acetate increased in the presence of melanoidins. This demonstrates that the melanoidins have a solvating effect. In most of the cases there is a weak influence of the melanoidin concentration on the flavor retention capacity, with a slight tendency to increase the retention with increasing melanoidin concentration. This tendency is best pronounced at an isoamyl acetate concentration of 10 ppm and 2 min extraction time. It is important to note that a short extraction time (30 s) mimicks best the conditions corresponding with a static headspace

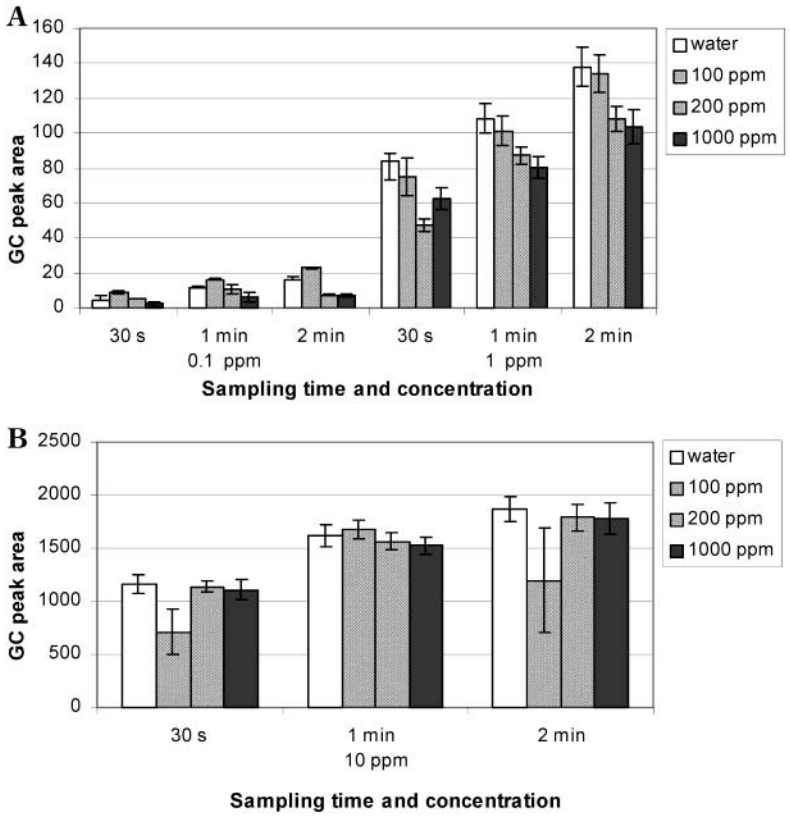

Figure 4. Isoamyl acetate retention capacity of melanoidins prepared from a vitamin C–Lys mixture (test in water and in aqueous solutions with melanoidin concentrations of 100, 200, and 1000 ppm): A, low (0.1 ppm) and moderate (1 ppm) concentration of isoamyl acetate; B, high concentration (10 ppm) of isoamyl acetate.

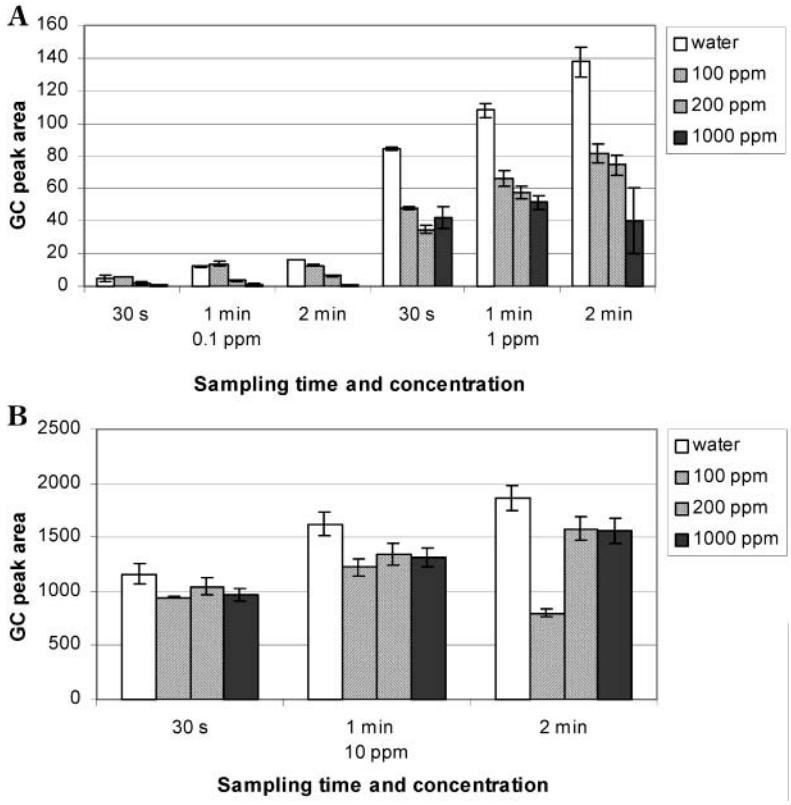

Figure 5. Isoamyl acetate retention capacity of melanoidins prepared from a vitamin C–Glu mixture (test in water and in aqueous solutions with melanoidin concentrations of 100, 200, and 1000 ppm): A, low (0.1 ppm) and moderate (1 ppm) concentration of isoamyl acetate; B, high concentration (10 ppm) of isoamyl acetate.

sample, whereas during longer extraction times (2 min) the equilibrium between the aqueous and headspace phases is influenced, resulting in a shift of the partition of the flavor compounds from the aqueous to the headspace phase.

Melanoidins derived from the L-(+)-ascorbic acid−glycine model system have a behavior similar to that of the above-mentioned melanoidins with respect to the isoamyl acetate retention capacity. In most solutions of the flavor compound (0.1 and 1 ppm) increasing melanoidin concentrations (100−200 ppm) increase the release of isoamyl acetate (**Figure 3**), demonstrating the solvating power of this melanoidin model system. Some flavor retention is observed with the highest melanoidin concentration (1000 ppm) in combination with moderate isoamyl acetate concentration (1 ppm). As in the previous case (**Figure 2**), some discrepancies are observed at 2-min extraction time, due to changes in the equilibrium between headspace and liquid phase, which can be different for every melanoidin concentration.

The effect of the melanoidins obtained from the L-(+)-ascorbic acid−lysine mixture on the release of isoamyl acetate is rather different from that of the melanoidins obtained from glucose/L-(+)-ascorbic acid−glycine. At 0.1 ppm concentration of isoamyl acetate, a low concentration (100 ppm) of melanoidins has a positive effect on the release of the aroma compound, demonstrated by the increased peak area. In almost all other cases (1 and 10 ppm of isoamyl acetate), however, the release of the flavor compound decreases with increasing melanoidin concentration, i.e. there is an interaction of isoamyl acetate with the melanoidin macromolecules (**Figure 4**). However, it is as yet not clear whether there is a real binding of the volatiles to the melanoidins or any other effect of sorption, because the possibility of inclusion of the odorant in cages of the macromolecule might also explain the analytical data.

A strong increase in the isoamyl acetate retention capacity of melanoidins is also observed in the presence of melanoidins prepared from L-(+)-ascorbic acid and glutamic acid (**Figure 5**). This influence is demonstrated in all the studied conditions but is most pronounced at an isoamyl acetate concentration of 1 ppm and an increasing melanoidin content.

Our results suggest that the influence of the α-amino acid participating in the generation of melanoidins (glycine, lysine, or glutamic acid) on the retention capacity of melanoidins is stronger than that of the carbonyl compound (glucose or L-(+)-ascorbic acid). The nonpolar amino acid (glycine, $pH_i = 5.97$) probably participates in the formation of low polar melanoidins that demonstrate mainly a solvating effect. The basic lysine ($pH_i = 9.74$) produces basic melanoidins, whereas glutamic acid ($pH_i = 3.22$) forms rather acidic melanoidins. It is suggested that these are the reasons for the higher retention capacity observed in the last two cases. There are possible dipole−dipole or dipole−ion ($-NH_3^+$, COO^-, etc.) type interactions between the melanoidins and isoamyl acetate.

Similar experiments were performed with 2-phenylethanol. This aroma compound, however, is only slightly soluble in water and has a very low volatility. Moreover, it could only be poorly extracted by SPME and therefore the release of this aroma compound alone was not further studied, but the investigation was focused on the effect of 2-phenylethanol as a second aroma compound in a multi-compound solution on the release of the model flavor compound, isoamyl acetate.

It can be concluded that SPME can be used for the study of the flavor release from melanoidin-containing solutions, provided the selected model flavor compound is volatile enough and slightly soluble in water. The reproducibility for isoamyl acetate extraction was good (5−10% RSD). The isoamyl acetate retention capacity of the studied melanoidins can be summarized as follows: the melanoidins prepared from L-(+)-ascorbic acid and glycine increased the release of isoamyl acetate, whereas the melanoidins prepared from L-(+)-ascorbic acid and lysine/glutamic acid caused a retention of the flavor compound; the melanoidins prepared from the glucose−glycine model system had the same effect as the melanoidins prepared from L-(+)-ascorbic acid−glycine.

In the food industry, the flavor retention capacity of the melanoidins is very important in products such as roasted and instant coffees, bread, beer, fruit juice, etc. Because of the flavor retention capacity of melanoidins, food products can maintain their organoleptic properties for a longer time.

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