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Formation and characterisation of melanoidin-like polycondensation products from amino acids and lipid oxidation products

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ABSTRACT

Various coloured water-soluble high molecular weight and water-nonsoluble reaction products were isolated from model reactions of an amino acid (glycine or lysine) and a lipid oxidation product (hexanal, (2*E*)-hexenal, (2*E*, 4*E*)-decadienal) with or without glucose. They were characterised by UV–visible absorbance measurements, elementary analysis, and thermal degradation followed by SPME–GC–MS analysis. The UV–visible absorbance spectra before and after dialysis indicated that the most important contributors to the formation of water-soluble coloured material were constituents of the low molecular fraction. Elementary analysis data indicated that a higher amount of nitrogen was incorporated in the high molecular weight fractions as compared to the water-nonsoluble fractions, except for the water-nonsoluble reaction products from amino acid/(2*E*, 4*E*)-decadienal interactions, which showed the lowest *C*/*N* ratio found. Volatile carbonyl compounds, furans, aliphatic compounds, pyridines, pyrroles and benzene derivatives were the main groups identified in the thermal degradation profile of each fraction tested. Especially pyridines seem typical indicators of amino acid–lipid oxidation product interactions.

1. Introduction

The Maillard reaction, or nonenzymatic browning, is one of the most widespread reactions occurring under thermal food treatment conditions, traditionally attributed to reducing sugar and amine compound interactions. The latest investigations in this field led to the conclusion that nonenzymatic browning is a much more complex process, because primary Maillard products can also react with endogenous food ingredients like lipids, flavonoids, terpenes and fermentation or metabolic products (Nursten, 2005).

The presence of lipid oxidation products in the classical Maillard reaction pathway and vice versa is of particular interest nowadays, as it would be unlikely in normal foods if either of these reactions would occur in isolation from the other. Due to the parallel modifications induced by both reactions in food colour (Hutapea et al., 2004), flavour and off-flavour (Gandemer, 1999; Whitfield, 1992), antioxidant activity, nutritional value and safety (Alaiz, Zamora, & Hidalgo, 1996; Cämmerer, 2000; Lee & Shibamoto, 2002), it is expected that the course of both reactions can be modified by the reactants, intermediates and products of the other (Zamora & Hidalgo, 2005).

Despite a completely different nature at first sight, both the Maillard reaction and lipid oxidation follow parallel fundamental 'three-step' mechanisms. In a first step, the key compounds (Amadori compounds in the Maillard reaction and lipid hydroperoxides in lipid oxidation) are formed. Secondly, fragmentation, rearrangement and degradation reactions of these key compounds produce a number of low molecular volatile and non-volatile monomers. Finally, the monomers from the second step reactions are responsible for the production of melanoidin-like coloured polycondensation products by aldol condensation, carbonyl–amine polymerisation and/or pyrrole polymerisation mechanisms (Hutapea et al., 2004; Zamora & Hidalgo, 2005).

The nature of the end products – flavour compounds as well as coloured macromolecular polymers – varies strongly according to the nature and quantity of the starting nitrogen and carbonyl compounds involved and the reaction conditions applied. Thus, no single melanoidin and/or melanoidin-like polycondensation product from oxidised lipids has been fully described yet and the formation, separation, and characterisation of these compounds still remain of particular interest.

Due to the complexity and heterogeneous nature of real food systems, the formation of melanoidin-like polycondensation products is mainly studied in model systems of an amino acid and a carbonyl compound. The choice of the initial reactants and preparation conditions for this study was based on a standardised protocol for the preparation of standard melanoidins, established in the European Research Programme COST Action 919 ('Melanoidins

Abbreviations: Dcdnl, (2*E*, 4*E*)-decadienal; Glc, D(+)-glucose; Gly, glycine; Hexa, hexanal; Hexe, (2*E*)-hexenal; HMW, high molecular weight; LMW, low molecular weight; Lys, L(+)-lysine; WNS, water-nonsoluble and hexane-nonsoluble.

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in Food and Health') (Ames et al., 2000), in order to enable the comparison of the results obtained with literature data. Glycine is structurally the simplest amino acid and is ubiquitous in nature and was, therefore, chosen as the amino compound to prepare standard COST melanoidins (Ames et al., 2000). L(+)-Lysine is an essential amino acid and, due to the presence of the ε-amino function, it is also one of the most reactive amino acids, when incorporated in a protein. Saturated and unsaturated aldehydes are important lipid oxidation products that are able to participate in amino acid–carbonyl compound interactions. Hexanal and (2*E*, 4*E*)-decadienal are quantitatively the most important decomposition products identified upon autoxidation of linoleic acid and its esters (Henderson, Witchwoot, & Nawar, 1980). In this study, three aldehydes with different degrees of unsaturation, i.e., hexanal, (2*E*)-hexenal and (2*E*, 4*E*)-decadienal, were selected for the model reactions with glycine or L(+)-lysine. D(+)-Glucose was added in some cases to the amino acid/lipid oxidation product model systems to study the interaction of amino acid/oxidised lipid and amino acid/carbohydrate reactions or the coordinate contribution of both pathways to the browning reactions. A temperature of 125 °C and a heating time of 2 h under water-free reaction conditions were chosen as representative for several common food processes (roasting, sterilisation, etc.) (Ames et al., 2000).

Various analytical methods, namely UV–visible absorbance, elementary analysis and thermal degradation (250 °C, 10 min) followed by SPME–GC–MS analysis (Abbaspour Tehrani, Keršienė, Adams, Venskutonis, & De Kimpe, 2002) were combined to characterise the different fractions of the coloured polycondensation reaction products thus obtained.

2. Materials and methods

2.1. Materials

Model reaction products were prepared using glycine (>99%, Sigma–Aldrich, Bornem, Belgium), L(+)-lysine monohydrochloride (>99%, Sigma–Aldrich), hexanal (>98%, Sigma–Aldrich), (2*E*)-hexenal (>99%, Janssen Chimica, Geel, Belgium), (2*E*, 4*E*)-decadienal (>95%, Acros Organics, Geel, Belgium), and D(+)-glucose, anhydrous (>99%, Fluka Biochemica, Bornem, Belgium). Dialysis tubing (average flat width – 33 mm; average diameter – 21 mm; capacity – ±360 mL/m, Sigma–Aldrich) was prepared according to the manufacturer's instructions. This cellulose membrane retains more than 90% cytochrome *c* (MW = 12,000 or greater) in solution over a 10 h period.

2.2. Preparation of melanoidin-like polycondensation products

In a 500 mL flask, 0.05 mol of amino acid, 0.025 mol of lipid oxidation product and 0.05 mol of D(+)-glucose (Table 1) were well-mixed and placed in an oven (equipped with a fan, Memmert, Schwabach, Germany), preheated to and stabilised at 125 °C (or 200 °C). The reaction mixtures were heated in anhydrous medium for exactly 2 h without covering. After heating, the flask with brown–brown to black coloured reaction products was placed in a desiccator to cool down to room temperature. The solid material was transferred to a mortar and grinded to a fine powder using a pestle. For each 5 g of the ground material, 200 mL of distilled water was added and the solution was stirred for 12 h to dissolve as much material as possible. The suspension was filtered through Whatman No. 4 filter paper, and the filtrate, containing the water-soluble reaction products, was collected. Residues on the filter paper (water-nonsoluble reaction products) were additionally washed with two times 20 mL of distilled water. These washings were mixed together with the original filtrate (Solution A). Next, 50–70 mL of Solution A was placed in the dialysis tubing and dialysed against 1 L of distilled water for 24 h at 4 °C with minimum two changes of the surrounding water. The dialysate, containing low molecular weight (LMW) compounds, was not collected. The contents of the dialysis tubings with the non-dialyzable high molecular weight reaction products (HMW) were transferred to a 500 mL round-bottom flask, frozen in a liquid nitrogen bath, and freeze-dried (−50 °C) until all the water was removed. The lyophilised HMW water-soluble reaction products were stored in the freezer until further use. Completely dried water-nonsoluble reaction products were mixed with hexane and stirred for four times 24 h at room temperature, thus separating into hexane-soluble and hexane-nonsoluble reaction products (WNS). The latter ones were dried and kept in the freezer for further analysis.

2.3. Thermal degradation and solid-phase microextraction (SPME)

Silanised 4-mL SPME-vials (Supelco Inc., Bellefonte, USA) were filled with 50 mg of HMW or WNS reaction products, closed with PTFE-silicone septa and open top polypropylene (Supelco) caps and heated on a sand bath at a constant temperature of 250 °C (±5 °C) for exactly 10 min. After cooling of the vials to room temperature, the headspace of the heated reaction products was extracted by means of solid-phase microextraction, using a DVB/carboxen/PDMS fibre (Supelco) during 5 min. Whenever sufficient material was available, the experiments were performed in duplicate.

2.4. Mass spectrometry

For the analysis of the SPME-extracts, an Agilent 6890 GC Plus (Agilent Technologies, Diegem, Belgium) coupled with an Agilent 5973 MSD (mass selective detector-quadrupole type), equipped with CIS-4 PTV (programmed temperature vapourisation) Injector (Gerstel, Mülheim an der Ruhr, Germany), and a AT5-MS capillary column (30 × 0.25 mm id; coating thickness 0.25 μm) was used.

Table 1
Composition of the different model systems (mmol), prepared under water-free reaction conditions (125 °C, 120 min).

Model system	Amino acids		Lipid oxidation products			Monosaccharides
	L(+)-Lysine	Glycine	Hexanal	(2*E*)-Hexenal	(2*E*, 4*E*)-Decadienal	D(+)-Glucose
I	50		25			
IA	50		25			50
II	50			25		
IIA	50			25		50
III		50	25			
IIIA		50	25			50
IV		50		25		
IVA		50		25		50
V		50			25	
VI	50				25	

Working conditions were: injector 250 °C, transfer line to MSD: 260 °C, oven temperature: start 40 °C, hold 2 min; programmed from 40 to 200 °C at 4 °C min^{-1} and from 200 to 240 °C at 30 °C min^{-1}, hold 2 min; carrier gas (He) 1.2 mL min^{-1}; splitless; ionisation EI 70 eV; acquisition parameters: scanned m/z 40–200 (0–10 min), 40–300 (10–20 min), 40–400 (>20 min). Volatile substances were identified by comparison of their mass spectra and retention times with those of reference substances and by comparison with the Wiley (6th edition) and the NIST Mass Spectral Library (Version 1.6d, 1998), and particular literature data, if available. In cases, when only MS data were available, identifications were considered to be tentative.

2.5. Analysis of elementary composition

An exact amount of the HMW and WNS reaction products was weighed (between 1.5 and 2 mg) and the percentages of carbon (*C*), hydrogen (*H*) and nitrogen (*N*) incorporated were determined with a CHNS/O Analyser 2400, PerkinElmer Series II (PerkinElmer, Zaventem, Belgium) operated in the CHN mode.

2.6. Spectophotometric measurements

UV–visible absorbance spectra of reaction products before (10^{-2} dilution of Solution A) and after dialysis (10^{-2} dilution of dialysis retentate) were obtained. Absorbances were recorded with a Cary 50 UV–visible Spectrophotometer (Varian, Sint–Katelijne–Waver, Belgium), using UV-Grade Silica Type 21 Cells (10 mm), at wavelengths ranging from 200 to 800 nm.

3. Results and discussion

3.1. Coloured polycondensation reaction products from hexanal and (2*E*)-hexenal containing model mixtures

Light to dark brown reaction products were obtained from amino acid/lipid oxidation product (molar ratio of 2:1) and amino acid/lipid oxidation product/D(+)-glucose (molar ratio of 2:1:2) interactions in an anhydrous medium at 125 °C (Table 1). The water-soluble reaction products were fractionated by dialysis (MW cut-off 12,000) to isolate the high molecular weight (HMW) fraction. The aqueous low molecular weight (LMW) fraction was not further studied in this work. The volatiles produced in these model systems will be reported elsewhere. Attempts were undertaken to dissolve the water-nonsoluble fraction in various solvents (methanol, ethanol, dichloromethane, diethyl ether, pentane and hexane). Hexane was the most effective solvent for these reaction products. The water-nonsoluble reaction products from the lysine/hexanal(I) and from the glycine/hexanal(III) model systems were completely soluble in hexane, while the water-nonsoluble fraction from the glycine/(2*E*)-hexenal(IV) model mixture was only partly soluble in hexane. The water-nonsoluble reaction products from other model systems were neither soluble in hexane nor in the other tested organic solvents. By means of this hexane extraction mainly long chain alkanes and some long chain carbonyl compounds were separated from the model mixtures.

Yields of the reaction products before and after dialysis varied according to the composition of the model system (Table 2). In the model reactions of glycine or lysine with hexanal or (2*E*)-hexenal, the weight loss after heating was small: in case of lysine, about 91% of all mass was recovered, while for glycine, this was around 85%. The differences in yield between hexanal and (2*E*)-hexenal model systems were small. In all cases, however, only a small fraction obtained after heating of the amino acid/carbonyl compound model mixtures consisted of water-soluble HMW reaction products (0.09–4.66 %). However, also for glucose/glycine model melanoidins such low yields of HMW water-soluble melanoidins have been reported (0.47%) (Obretenov et al., 2002). The weight loss during heating (production of volatiles) was, in general, higher for the glycine model systems than for the lysine model systems. The addition of glucose to the model systems also imparted a considerably higher weight loss during heating. Similar trends were observed for the HMW fraction: higher yields were generally obtained in the presence of glycine as compared to lysine (except for the amino acid/(2*E*)-hexenal model system) and in the presence of glucose as compared to in the absence of glucose. These results imply that glycine was more reactive than lysine and that the addition of glucose accelerated the reaction. Heating of the lysine/hexanal and lysine/(2*E*)-hexenal model systems at 200 °C instead of 125 °C resulted in a higher weight loss (yields before dialysis 83% and 85% for hexanal and (2*E*)-hexenal model systems, respectively). The yields of HMW compounds were also higher, being 0.52% and 4.3% for hexanal and (2*E*)-hexenal model systems, respectively.

Table 2
Yields of water-soluble reaction products from the different model systems prepared under water-free reaction conditions (125 °C, 120 min) before and after dialysis.

Model system	Yield (%) before dialysis (LMW + HMW)	Yield (%) of HMW compounds after dialysis
I (Lys/Hexa)[a]	90.6	0.09
IA (Lys/Hexa/Glc)	71.7	0.39
II (Lys/Hexe)	91.9	0.26
IIA (Lys/Hexe/Glc)	76.9	2.57
III (Gly/Hexa)	84.7	0.32
IIIA (Gly/Hexa/Glc)	63.6	0.85
IV (Gly/Hexe)	85.7	0.16
IVA (Gly/Hexe/Glc)	69.3	4.66

[a] 'Lys', L(+)-lysine; 'Gly', glycine; 'Hexa', hexanal; 'Hexe', (2*E*)-hexenal; and 'Glc', D(+)-glucose.

3.1.1. UV–visible absorbance measurements

The water-soluble fractions of the final reaction products were characterised by obtaining the UV–visible absorbance spectra, at wavelengths ranging from 200 to 800 nm, before and after the dialysis procedure (Ames et al., 2000). The absorbance values of the different fractions at 280, 360 and 420 nm are depicted in Table 3. In all cases, the UV–visible absorbance spectra demonstrated a strong absorbance at the intermediate wavelengths (λ_{max} between 260 and 280 nm) which gradually decreased as the wavelength increased. Besides, all HMW fractions showed a lower absorbance than the water-soluble reaction products before dialysis (LMW + HMW). Thus, the most important contributors to the formation of water-soluble coloured material seem to be of low molecular weight (<12,000), quantitatively as well as qualitatively. Similar characteristic features and shape of the UV–visible absorbance spectra have been reported to be typical for melanoidins (Clark & Tannenbaum, 1970; Hofmann, 1998). Also in the reaction of glucose with amino acids, the main contributors to the brown colour of the reaction mixture were of low molecular weight (Hofmann, 1998).

The measured UV–visible absorbance values at the specific wavelengths varied according to the composition of the model systems. In general, higher absorbance values were recorded in the presence of glycine as compared to lysine (except for the (2*E*)-hexenal model system). The presence of glucose led to a considerable increase of the absorbance values of all tested water-soluble reaction products (except for the lysine/hexanal HMW fraction). These results confirm the hypothesis that glycine is more reactive in the browning reaction than lysine and that the presence of glucose induced the browning reactions. As compared to the absorbance

Table 3
Results of UV–visible absorbance (10^{-2} dilution) of the water-soluble reaction products of the different model systems, before and after dialysis.

Model system	Abs before dialysis (LMW + HMW)			Abs of HMW compounds after dialysis		
	280 nm	360 nm	420 nm	280 nm	360 nm	420 nm
I (Lys/Hexa)[a]	0.08	0.02	0.01	0.02	0.02	0.01
IA (Lys/Hexa/Glc)	0.72	0.36	0.27	0.01	0.01	0.01
II (Lys/Hexe)	0.14	0.03	0.02	0.03	0.01	0.01
IIA (Lys/Hexe/Glc)	0.66	0.29	0.27	0.20	0.05	0.04
III (Gly/Hexa)	0.27	0.02	0.02	0.10	0.00	0.00
IIIA (Gly/Hexa/Glc)	0.94	0.26	0.07	0.19	0.05	0.01
IV (Gly/Hexe)	0.07	0.02	0.01	0.15	0.01	0.00
IVA (Gly/Hexe/Glc)	2.01	0.65	0.28	0.31	0.18	0.10

[a] 'Lys', L(+)-lysine; 'Gly', glycine; 'Hexa', hexanal; 'Hexe', (2*E*)-hexenal; and 'Glc', D(+)-glucose.

values recorded for glucose/glycine melanoidins (Obretenov et al., 2002) the values for glycine/lipid oxidation product polycondensation products were lower but the absorbance of polycondensation products resulting from glycine/lipid oxidation product/glucose interactions were higher, especially of the glycine/(2*E*)-hexenal/glucose model system.

Heating of the model systems (lysine/hexanal and lysine/(2*E*)-hexenal) at 200 °C instead of 125 °C resulted in higher absorbance values of the water-soluble fractions (data not shown).

3.1.2. Analysis of elementary composition

Table 4 outlines the percentage of carbon, hydrogen and nitrogen incorporated into the structure of the HMW and WNS fractions of the different model systems. In addition, the *C*/*N* ratio was calculated for each fraction available. In general, reaction products from the (2*E*)-hexenal-containing model systems showed a higher *C*/*N* ratio than comparable products from hexanal-containing model systems. Most probably, (2*E*)-hexenal is more sensitive to self-condensation by means of aldol condensations, which leads to the formation of compounds with a higher carbon, but lower nitrogen content. Comparing the elementary composition of two fractions of the same model system, a higher percentage of carbon was incorporated in the WNS fraction than in the corresponding HMW fraction. Addition of glucose to the model systems increased the *C*/*N* ratio for the HMW fractions, while the *C*/*N* ratio of the WNS fractions was lower in the presence of glucose (although not enough data are available to draw reliable conclusions on this point). The influence of the amino acid (glycine or lysine) on the incorporation of nitrogen in the corresponding HMW and WNS fraction is not clear.

Previous studies of the elementary composition of water-soluble HMW model melanoidins from various carbohydrates with glycine (180 °C, 10 min) showed *C*/*N* ratios between 7.4 and 26.3 (Cämmerer & Kroh, 1995). That study also showed that the molecular composition of the model melanoidins was only negligibly influenced by the molar ratio of the reactants, but was highly influenced by the reaction conditions. Comparison of these results with our study shows that, in general, higher amounts of nitrogen were incorporated in the polycondensation products resulting from glycine/lipid oxidation product interactions and, thus, lower *C*/*N* ratios were obtained. This may be a result of the higher reactivity and polymerisation capacity of carbohydrates and their degradation products as compared to long chain aldehydes, although the different reaction conditions applied hamper a clear comparison of the results. In agreement with the same study, heating of the model systems (lysine/hexanal and lysine/(2*E*)-hexenal) at a higher temperature (200 °C instead of 125 °C) resulted in HMW water-soluble compounds with a higher *C*/*N* ratio (data not shown).

3.1.3. Thermal degradation studies

For the thermal degradation experiments, water-soluble high molecular weight (HMW) and water- and hexane-nonsoluble (WNS) reaction products were heated at 250 °C for 10 min, after which the headspace was sampled by means of solid-phase microextraction (SPME) for 5 min. Not all the model reaction products prepared were analysed in this way, since not enough material was available (<50 mg). It has been shown before that the thermal degradation profile thus obtained is useful for the characterisation of different types of melanoidins and gives information on the flavour compounds generated from melanoidin degradation (Adams, Abbaspour Tehrani, Keršienė, Venskutonis, & De Kimpe, 2003). Upon thermal degradation of melanoidins isolated from tomato sauce, several compounds resulting from lipid oxidation processes were detected, i.e., hexanal, heptanal, nonanal, decanal and 2-pentylfuran (Adams, Borrelli, Fogliano, & De Kimpe, 2005). Identification of these compounds suggested that lipid oxidation products were incorporated in the high molecular weight melanoidin skeleton and brought about the present study.

Table 4
Elementary composition of the various reaction products of the different model systems, prepared under water-free reaction conditions (125 °C, 120 min).

Model system	*C* (%)		*H* (%)		*N* (%)		*C*/*N*	
	HMW	WNS	HMW	WNS	HMW	WNS	HMW	WNS
I (Lys/Hexa)[a]	37.7	NA[b]	7.8	NA	11.3	NA	3.3	NA
IA (Lys/Hexa/Glc)	54.1	56.5	6.8	6.8	6.6	7.3	8.2	7.7
II (Lys/Hexe)	52.3	68.6	8.3	8.9	7.7	4.1	6.8	16.9
IIA (Lys/Hexe/Glc)	55.2	54.9	5.7	7.3	6.5	7.8	8.5	7.1
III(Gly/Hexa)	NA	NA	NA	NA	NA	NA	NA	NA
IIIA (Gly/Hexa/Glc)	50.2	56.7	5.6	5.9	7.0	7.0	7.2	8.1
IV (Gly/Hexe)	NA	72.0	NA	9.7	NA	4.9	NA	14.6
IVA (Gly/Hexe/Glc)	52.0	57.9	5.8	6.4	5.2	4.8	10.0	12.0
V (Gly/Dcdnl)	NA	42.36	NA	7.40	NA	14.55	NA	2.9
VI (Lys/Dcdnl)	NA	48.00	NA	8.70	NA	11.82	NA	4.1

[a] 'Lys', L(+)-lysine; 'Gly', glycine; 'Hexa', hexanal; 'Hexe', (2*E*)-hexenal; 'Glc', D(+)-glucose; and 'Dcdnl', (2*E*, 4*E*)-decadienal.
[b] NA, not available.

On the basis of the chemical structure, the volatiles identified were divided in five main groups: volatile carbonyl compounds, nitrogen-containing (pyrroles, pyridines and pyrazines) and oxygen-containing heterocyclic compounds (furans), other substituted aromatic (benzene derivatives) and aliphatic (alkanes, alkenes and alkynes) compounds and alcohols (Supplementary Table 5). Fig. 1 represents the thermal degradation profiles of the different HMW and WNS fractions obtained. However, not all the compounds could be identified based on the mass spectrum and a relatively large part of the volatiles remained unknown.

The relatively high amounts of carbonyl compounds detected in the headspace of thermally destructed hexanal polycondensation products are mainly due to the release of hexanal (9.4–18.5%) and its aldol condensation product 2-butyl-2-octenal (1.2–15.2%). Apparently, hexanal was only reversibly bound in the high molecular weight fractions and was easily released again upon heating. The release was higher in case of water-nonsoluble reaction products. Release of (2*E*)-hexenal was much lower (0–4.6%), as can be expected due to the more reactive nature of this unsaturated aldehyde which is thus irreversibly incorporated. In addition, various saturated and unsaturated aldehydes, ketones, C_5 and C_6 carboxylic acids and their esters were detected in the headspace of the thermally destructed samples. These compounds may as such contribute to the overall aroma of fried lipid-containing foods, meaning the fatty, tallowy, waxy, oily, pungent, roasted, rancid notes (Mottram, 1998; Rochat & Chaintreau, 2005).

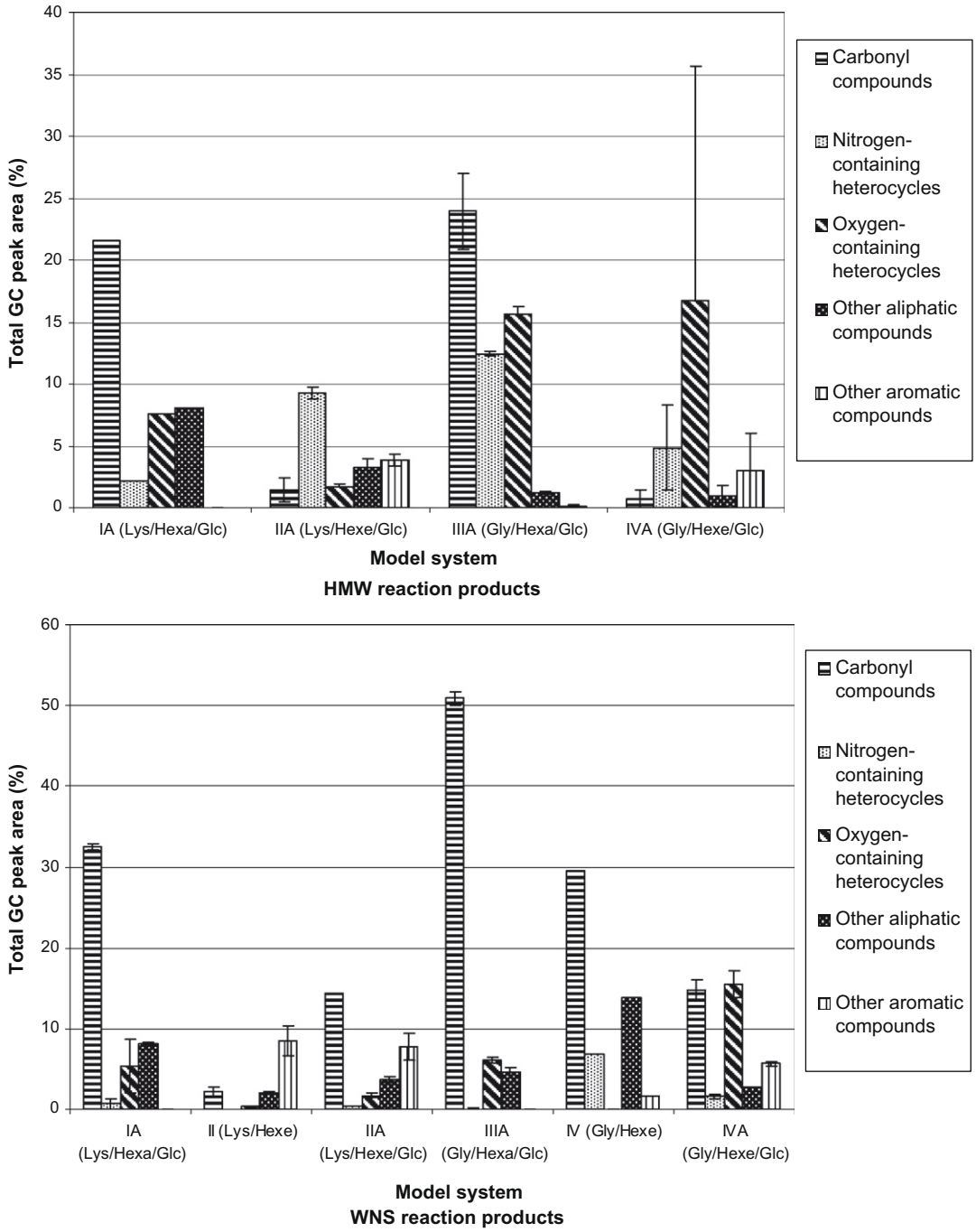

Fig. 1. Thermal degradation profiles (250 °C, 10 min) of different HMW and WNS reaction products from glycine (Gly) or lysine (Lys) with hexanal (Hexa) or (2*E*)-hexenal (Hexe) with or without glucose (Glc).

Thirty-one nitrogen-containing and sixteen oxygen-containing alkyl- and acyl-substituted heterocyclic aromatic compounds were detected in the headspace of the thermally destructed samples.

Pyrroles were mostly released from the HMW water-soluble fractions resulting from the interaction of glucose and glycine, with or without a lipid oxidation product. The main pyrroles identified, 1-methyl-1*H*-pyrrole, 1-methyl-1*H*-pyrrole-2-carbaldehyde and 4,5-dimethyl-1*H*-pyrrole-2-carbaldehyde correspond to the ones previously identified upon thermal degradation of standard glucose/glycine melanoidins (Adams et al., 2003). As such, the presence of hexanal or (2*E*)-hexenal does not contribute to pyrrole release after thermal degradation.

Tressl, Wondrak, Krüger, and Rewicki (1998) reported that various furan derivatives, *N*-substituted pyrroles and *N*-substituted pyrrole-2-carbaldehydes possess an extraordinary polycondensation activity and form possible structural units of the final Maillard reaction products. The detection of these compounds suggests that similar structures might take part in the complex macromolecular structures of the coloured polycondensation reaction products discussed here. *N*-Substituted 2-(1-hydroxyalkyl)pyrroles have been described as potential key intermediates of both the Maillard reaction and amino acid/oxidised lipid interactions (Zamora, Alaiz, & Hidalgo, 2000).

Seventeen alkyl- and acyl-substituted pyridines were formed from the heated HMW and WNS fractions. The highest amounts of pyridines were found after degradation of polycondensation products prepared with (2*E*)-hexenal. 5-Butyl-2-propylpyridine (up to 3.2%) and 3-ethylpyridine (up to 2.9%) were the major pyridine derivatives detected. 5-Butyl-2-propylpyridine can result from the condensation of two molecules of (2*E*)-hexenal with ammonia. Thermal degradation of standard glucose/glycine melanoidins yielded much lower amounts of pyridines, among which the parent pyridine dominated. This suggests that alkylpyridine formation can be primarily ascribed to the interaction of the lipid oxidation product ((2*E*)-hexenal) with *N*-containing compounds or ammonia. Several alkylated pyridines have also been identified after reaction of glycine with heated oils (Macku & Shibamoto, 1991; Wang, Wu, & Wu, 1999). Thermal degradation of the polycondensation products resulting from the lysine/hexanal and lysine/(2*E*)-hexenal model systems prepared at 200 °C showed an even higher pyridine formation. The share of pyridines in the headspace profile of the water-soluble HMW products amounted to 14.8% and 24.4% for hexanal and (2*E*)-hexenal model systems, respectively.

Only three alkyl-substituted pyrazine derivatives (methylpyrazine, 2-ethyl-6-methylpyrazine and 3-ethyl-2,5-dimethylpyrazine) were detected, mainly in the headspace of thermally destructed fractions from glucose-containing model systems. Heating of the model systems (lysine/hexanal and lysine/(2*E*)-hexenal) at 200 °C instead of 125 °C resulted in a higher release of pyrazines and pyridines from the HMW water-soluble compounds (data not shown).

The formation of furans upon heating of the polycondensation products under study resulted mainly from glucose-containing model systems. Quantitatively the most important furans (2-methylfuran, 2,5-dimethylfuran and especially 5-methylfurfural) were also the most abundant furans in standard glucose/glycine melanoidins. Thus, production upon heating was barely influenced by the presence of the aldehydes. The high deviation of the bar representing the oxygen-containing heterocycles in Fig. 1 is due to a very small peak of 5-methylfurfural noted in one of the replicate samples.

Alkanes, alkenes, alkynes and dienes are reported to result from both the Maillard reaction and lipid oxidation (Whitfield, 1992) and were detected in almost all thermally destructed HMW and WNS fractions. Various benzene derivatives were mainly detected in lysine and (2*E*)-hexenal containing model systems with or without glucose.

Similar heterocyclic aromatic compounds – furans, pyrroles, pyridines and pyrazines, with an exception of oxazoles – were detected in the thermal degradation profile of standard glucose/glycine melanoidins (Adams et al., 2003). Also in this case, higher levels of volatile heterocyclic aromatic compounds were typical for standard HMW melanoidins, while WNS fractions were characterised by larger amounts of volatile carbonyl compounds generated. In this sense, the model reaction products, especially from glycine/lipid oxidation product/glucose interactions, are similar to standard glucose/glycine melanoidins. On the other hand, differences were noted in the quantitative distribution of the volatiles identified. For example, the thermal degradation profile of standard HMW and WNS glucose/glycine melanoidins yielded mainly furans (54.8% and 71.6%, respectively). Similarly, higher amounts of pyrroles (24 ± 7%) were released from standard HMW melanoidins as compared to the model reaction products tested for the purposes of this study. On the other hand, pyridines mainly resulted from the amino acid/lipid oxidation product and amino acid/lipid oxidation product/glucose interactions. The relatively high amounts of carbonyl compounds and low amounts of pyrroles and furans formed upon thermal degradation of amino acid/lipid oxidation product polycondensation products suggest that aldol condensation reactions are very important in the polycondensation mechanisms of these fractions.

3.2. Coloured polycondensation reaction products from (2E, 4E)-decadienal containing model mixtures

Only water-nonsoluble reaction products were obtained from amino acid/(2*E*, 4*E*)-decadienal mixtures. According to the elementary analysis data, lysine/(2*E*, 4*E*)-decadienal (*C*/*N* ratio 4.1) and glycine/(2*E*, 4*E*)-decadienal (*C*/*N* ratio 2.9) derived WNS model reaction products incorporated considerably higher amounts of nitrogen as compared to the hexanal and (2*E*)-hexenal model systems studied (*C*/*N* ratios 7.1–16.9 for WNS fractions). The reaction products from the glycine/(2*E*, 4*E*)-decadienal model system showed a lower *C*/*N* ratio than the corresponding lysine/(2*E*, 4*E*)-decadienal reaction products. The water- and hexane-nonsoluble fractions were thermally destructed (250 °C, 10 min) and analysed by means of SPME–GC–MS (Supplementary Table 6).

Volatile carbonyl compounds, heterocyclic aromatic compounds, and aromatic and aliphatic compounds (alkanes, alkenes and alkynes) compose the thermal degradation profile of the WNS fractions from amino acid/(2*E*, 4*E*)-decadienal model mixtures (Fig. 2).

Volatile carbonyl compounds (especially 2-heptanone and 2-hexanone) were mainly formed from the lysine/(2*E*, 4*E*)-decadienal model mixtures.

Among the volatile azaheterocycles identified, pyridines were the most important group, originating in comparable quantities from glycine and lysine/(2*E*, 4*E*)-decadienal model mixtures. Among these, 2-pentylpyridine is of interest. This compound was released in a relatively large quantity (2.7–3.8%), in comparison with the other volatile azaheterocycles detected, and was reported to possess a fatty, tallowy-like odour and a very low threshold value (Kim, Hartman, & Ho, 1996). Its formation results from the reaction of (2*E*, 4*E*)-decadienal with either free ammonia (released from the amino acids) or with the α-amino group bound in the amino acids (Kim et al., 1996).

Thermal destruction of the WNS reaction products from the glycine/(2*E*, 4*E*)-decadienal reaction mixture resulted in the identification of five alkyl-substituted 1*H*-pyrrole derivatives. On the contrary, no pyrroles were detected in the headspace of heated WNS reaction products from the lysine/(2*E*, 4*E*)-decadienal lipid oxidation product mixtures, as was the case for the reaction products of lysine/hexanal and (2*E*)-hexenal reaction mixtures.

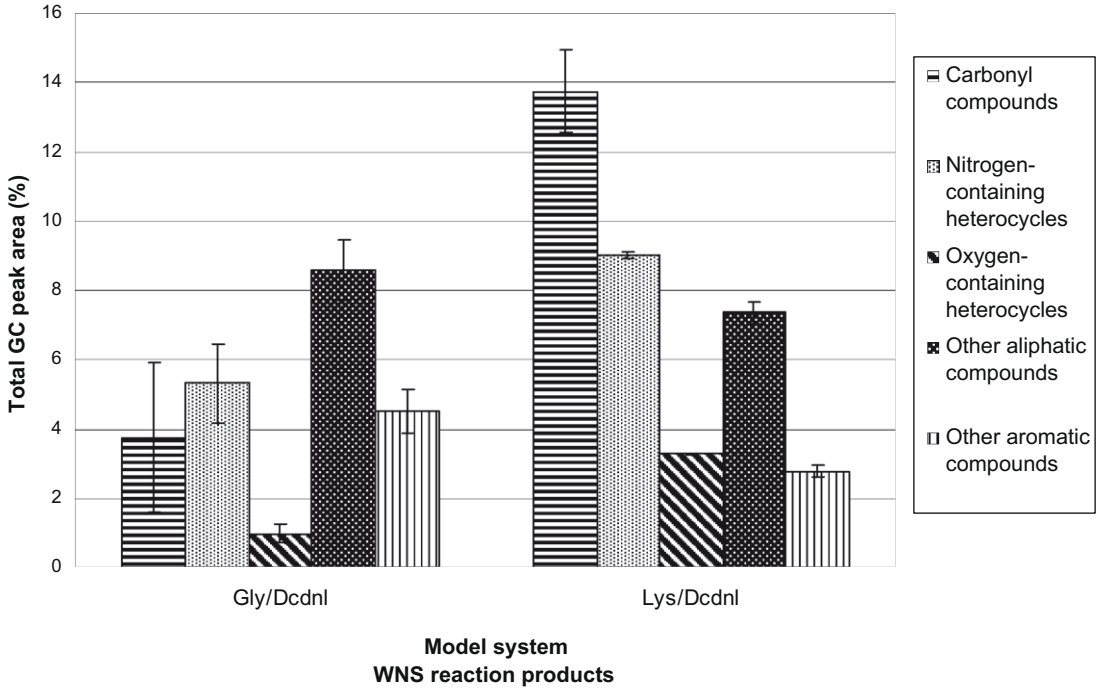

Fig. 2. Thermal degradation profiles (250 °C, 10 min) of the WNS reaction products from glycine (Gly) or lysine (Lys) with (2*E*, 4*E*)-decadienal (Dcdnl).

2-Pentylfuran was the only volatile oxygen-containing heterocyclic compound identified.

Relatively high amounts of volatile alkanes, alkenes and alkynes were detected in the headspace of the WNS (2*E*, 4*E*)-decadienal reaction products.

4. Conclusion

Relatively low amounts of HMW brown-coloured polycondensation products were isolated from model reactions of amino acids with aldehydes resulting from lipid oxidation, in the presence or absence from glucose. The nature of the polycondensation products can be compared with melanoidins resulting from amino acid–glucose interactions. Although the incorporation of nitrogen in the HMW structures was lower, relatively more pyridines were formed upon thermal degradation. More aliphatic, aromatic and long chain carbonyl compounds were recovered upon heating, while less furans, pyrroles and pyrazines were formed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2009.01.005. Tables 5 and 6, listing the identities and GC peak areas of all the volatiles produced upon thermal degradation.

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