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Purification and Partial Characterization of Melanoidins Fractions from Toasted Oak Heartwood, Comparison with Melanoidins from Roasted Coffee

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Abstract

During the cooking, processing, and storage of food products, a whole range of browning reactions occurs, initiated by the reaction of a carbohydrate with a compound possessing a free amino group. Melanoidins formed, influence food quality, mainly their colour, their flavour, and their antioxidant activities. Melanoidins are complex Maillard reaction products. We developed a method to isolate coffee melanoidins and melanoidins from toasted oak wood. We noted that coffee is richer in melanoidin compounds than oak wood. We presented a partial characterization of melanoidins fractions from toasted oak heartwood, and a comparison with melanoidins from roasted coffee. Mass spectra of the fractions isolated from toasted oak wood indicate the presence of pentose and hexose-based oligosaccharides with different degrees of polymerisation. The presence of the oligosaccharide moieties, as well as their degradation products found in the oak wood melanoidins, supports the postulated carbohydrate-based origin of melanoidins.

Keywords: oak wood, melanoidins, LC/MS, NMR, IR

1. Introduction

During the cooking, processing, and storage of food products, a whole range of browning reactions occurs, initiated by the reaction of a carbohydrate with a compound possessing a free amino group. Melanoidins occur in many stored and processed foods. They influence food quality, mainly owing to their colour, their flavour, their antioxidant activities (Manzocco et al., 2001; Morales et al., 2005; Lindenmeier et al., 2002; Charles-Bernard et al., 2005; Ames, 1998; Delgado-Andrade & Morales, 2005; Delgado-Andrade et al., 2005; Wang et al., 2011; Borrelli et al., 2002; Andriot et al., 2004). Melanoidins are present in several food products, such as coffee, roasted malt, breakfast cereals, bread…

Melanoidins are complex Maillard reaction products. Melanoidins are formed from cyclizations, dehydrations, retroaldolizations, rearrangements from nitrogen compounds and sugars, isomerizations, and condensations of low molecular weight Maillard reaction products. Due to the high reactivity of the intermediates, a complex polymerization takes place, resulting in brown-colored high molecular weight melanoidins. The composition of melanoidin chemical structures is relatively unknown, however, due to the complexity of the products that are generated in the reaction (Bekedam et al., 2008; Kim & Lee, 2009). Based on model systems, different suggestions have been made for melanoidins structures (Tressl et al., 1998; Hofmann, 1988; Cämmerer et al., 2002; Nunes & Coimbra, 2007). However, their complexity and structures depend on the nature and number of possible reactants and the reaction conditions. Therefore the Maillard reaction in foods and the structures of the resulting melanoidins are presumed to be much more complex than in model systems, and different melanoidin structures may coexist (Adams et al., 2005). Different hypotheses have been formulated on the structural backbone of melanoidins. A first hypothesis states that a melanoidin skeleton is constituted mainly from sugar degradation products, polymerized through aldol-type condensations and probably branched via amino

compounds (Kato & Tsuchida, 1981; Yaylayan & Kaminsky; 1998; Cämmerer & Kroh; 1995). Tressl et al. proposed a complex macromolecular structure consisting of repeating units of furans and pyrroles, linked by polycondensation reactions. Hofmann et al. identified low-molecular weight chromophores and postulated the generation of melanoidin-type colorants by a cross-linking reaction between these low molecular weight substances and noncolored high molecular weight biopolymers, such as proteins.

One of the strategies in melanoidin characterization is the chemical or thermal degradation of melanoidins,[18-19] followed by the identification of the decomposition products formed, giving information on structural domains of the melanoidin network. In addition, thermal destruction of melanoidins leads to the formation of volatiles that contribute to the development of aroma in roasted food systems (Kuntcheva et al., 1998; Adams et al., 2005; Adams et al., 2003).

In coffee matrix the amount of melanoidins is often represented 25% of the dry matter. During toasting of oak wood used in making barrels, melanoidins are formed. The aim of this study was to develop a method to isolate coffee melanoidins and melanoidins from toasted oak wood. We compared melanoidins from coffee and from toasted oak wood by different analytical techniques: absorption spectra, IR, viscosity, headspace/GC/MS, elemental analysis, LC/ESI/MS, NMR.

2. Material and Methods

2.1 Materials

Different toasted oak wood were tested: the first one was a no toasted oak wood, and the others, oak wood with different degree of toasting.

2.2 Isolation of Coffee Melanoidins

According to the technique described by Hofmann et al., coffee (10 g) was extracted with hot pure water (80-90 ℃) until no coloured material could be extracted. The aqueous phase was extracted twice with dichloromethane (200 ml) to eliminate lipids and then freeze-dried. The obtained fraction was the Total Fraction Melanoidins coffee (TFMC).

We used HPLC/DAD technique in order to separate purified compounds. The compounds were collected and the different purified fractions were dried and frozen.

2.3 Isolation of Oak Wood Melanoidins

According to Hofmann et al. (2001) in the case of isolation of coffee melanoidins, oak wood (10 g) was extracted with hot pure water (80-90 ℃) until no coloured material could be extracted. The aqueous phase was extracted twice with dichloromethane (200 ml) to eliminate lipids and then freeze-dried. The obtained fraction was the Total Fraction Melanoidins Wood (TFMW).

We used different analytical techniques in order to separate purified compounds. First technique consisted to optimize the HPLC/DAD conditions. The compounds were collected and the different purified fractions were dried and frozen. The second one consisted to optimize low pressure gel chromatography: choice of gel, solvents, flow in order to separe and collected purify fractions; we used UV at 280 nm for detection. Fractions were obtained in quantity more important than in the case of liquid chromatography.

2.4 HPLC/DAD Analysis

HPLC/UV-Visible analyses were performed with a Waters separation module system, a Waters UV-Visible detector, and Millenium32 chromatography manager software. UV-visible spectra were recorded at 280 nm and 260 nm. The column was a reverse-phase Sunfire C18 (5 µm packing, 250 x 5 mm i.d.) protected with a guard column of the same material; solvent A, water/formic acid (98:2, v/v); solvent B, acetonitrile/water/formic acid (80:18:2, v/v). The column was placed at ambient temperature (T=21 ℃). The elution program was performed at a constant flow of 1 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 20 µl.

2.5 Semi-preparative HPLC

The fractions were collected and purified by semi preparative HPLC. Analyses were performed with a Waters separation module system, a Waters UV-Visible detector, and Millenium[32] chromatography manager software. UV-visible spectra were recorded at 280 nm and 260 nm. The column was a reverse-phase Sunfire Prep C18 (5 µm packing, 250 x 10 mm i.d.) protected with a guard column of the same material; solvent A, water/formic acid (98:2, v/v); solvent B, acetonitrile/water/formic acid (80:18:2, v/v). The column was placed at ambient

temperature (T=21 ℃). The elution program was performed at a constant flow of 3 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 200l.

2.6 Low Pressure Gel Chromatography

Separation was performer using low pressure TSK HW-40(S) gel chromatography with 100% H_2O and then 100% MeOH as eluents. Flow rate: 0,8 mL/min; column : 27 ±2 cm length and 2,5 cm diameter.

2.7 LC/ESI/MS Analysis

LC/ESI/MS, LC/ESI/MS/MS and ESI/HR/MS were performed on a Q-Star™ instrument using an electrospray ionization source in negative-ion mode. Ion spray voltage was selected at 4.5 KV, the capillary temperature was selected at 275 ℃, and the source temperature was selected at 400 ℃. The values of sheath gas flow rate were: nebulisation gas=2.85 l/min, turbo gas=4.8 l/min, curtain gas=1.48 l/min. For MS/MS measurements, the energy collision and the gas collision were respectively –30 eV and 5. Isolation window was 1 uma. For HR/MS analysis, we used an internal calibration using PPG. The resolution obtained was 12 000.

The column was a reverse-phase Interchim C18 (10 μm packing, 250 x 4.7 mm i.d.) protected with a guard column of the same material; solvent A, water/acetic acid (98:2, v/v); solvent B, acetonitrile/water/acetic acid (80:18:2, v/v). The column was placed at ambient temperature (T=21 ℃). The elution program was performed at a constant flow of 1 ml/min using the LC inlet and splitter outlet connection permitting to reduce the flow at 0.5 ml/min, passing from 5 to 30% of B in 40 min., and then rising to 40% of B in 10 min., and finally to 100% of B in 5 min., followed by washing and re-equilibrating the column during 15 min. The injection volume was 20 μl.

2.8 Absorption Spectra

Spectrophotometric measurements were performed using an Anthelie Secomam™ spectrophotometer and UV-Visible spectra were recorded with a spectrophotometer fitted with a quartz cell (1 cm). The wine-like samples were diluted (1/20) and then their spectra measurements were taken.

2.9 IR

The Spectrum was recorded with a Nicolet iS50 FT-IR Fourier Transform Infrared Spectrometer (Thermo Scientific, Madison, WI) equipped with a DTGS/KBr detector, KBr beamsplitter and an iS50-ATR diamond accessory. Each spectrum is obtained from the accumulation of 500 scans using an aperture of 150 at 45 ° of incidence from 4000 to 400 cm-1 with a 4 cm-1 resolution, and an Happ-Genzel apodisation. Then spectrum was treated for ATR correction, thereafter a small offset was applied for baseline. Attributions were done taking in account the formula $C_{24}H_{49}O_{23}N$, references, tables and softwares (Omnic, KnowItAll).

2.10 Headspace/GC/MS

The total fraction of melanoidins (TFMC and TFMW) obtained from toasted oak wood and coffee were heated at 250 ℃ (10 min), and the produced volatiles were analyzed by SPME-GC-MS. For the analysis of the SPME extracts a Thermo coupled with, and a HP5-MS column (30m x 0.25 mm i.d., 0.25 μm) was used. Working conditions were as follows: injector, 250 ℃; transfer line, 250 ℃; oven temperature, start 40 ℃ and hold 2 min, programmed from 40 to 120 ℃ at 4 ℃/min and from 120 to 240 ℃ at 30 ℃/min, hold 2 min; carrier gas (He), 1.2 ml/min; splitless; ionization EI, 70 eV; acquisition parameters, scanned m/z 40-600. Compounds were identified by comparison of their mass spectra and retention times with those of reference compounds and by comparison with the NIST Mass Spectral Library. When only MS data were available, identities were considered to be tentative.

2.11 NMR Experiments

NMR experiments were performed at 298 K using a Bruker Avance 800 MHz spectrometer equipped with a 5 mm TCI (^1H / ^{13}C / ^{15}N/ ^2H) cryoprobe with Z-gradients and ATM accessory. All ^1H NMR and ^{13}C chemical shifts are given with respect to tretramethylsilane in D_2O as an external reference.

^1H NMR spectra was acquired with water suppression using excitation sculpting (pulsed field gradients applied for 3 ms). 1D ^1H spectra was acquired with 8 transients were typically collected at frequency of 800.23 MHz with a flip angle of 90 ° (7.5 μs), a spectral width of 10000 Hz, acquisition time of 1.64 s and 32 K data points. Data were processed with multiplication prior to Fourier transformation by an exponential function (line broadening was 0.3 Hz) and with zero filling.

^{13}C NMR spectra. 1D ^{13}C spectra was acquired with power gated decoupling using 30 ° flip angle, spectral widths of 48 kHz consisting of 64 K data points, acquisition time of 0.68 s. Data were processed with multiplication

prior to Fourier transformation by an exponential function (line broadening was 2 Hz) and with zero filling.

2.12 Elemental Analysis

The analyzes were performed on an elemental analyzer Thermo Finnigan Flash EA 2000 equipped with an autosampler of 32 samples and a chromatographic column. The system is managed by the Eager 300 software Metered elements C, H, N, S and O. The results are provided with an absolute accuracy of ±0.2% and are valid for a minimum of two tests.

The combustion of the sample takes place at high temperature (940 ℃) in the presence of tungstic anhydride (WO 3) in a stream of oxygen for a very short (15s). This decomposition gives CO_2, H_2O, SO_2, NO_2, nitrogen oxides are reduced to N2 (nitrogen) with copper. The entire system is swept by a stream of helium.

For oxygen, pyrolysis of samples were performed under a stream of helium and gas chromatography with a gas-solid stationary phase compound formed CO. Because of the filling system of the reaction tube, the dosage of oxygen can be achieved with the samples containing fluorine.

3. Results

3.1 Isolation and Purification of Melanoidins

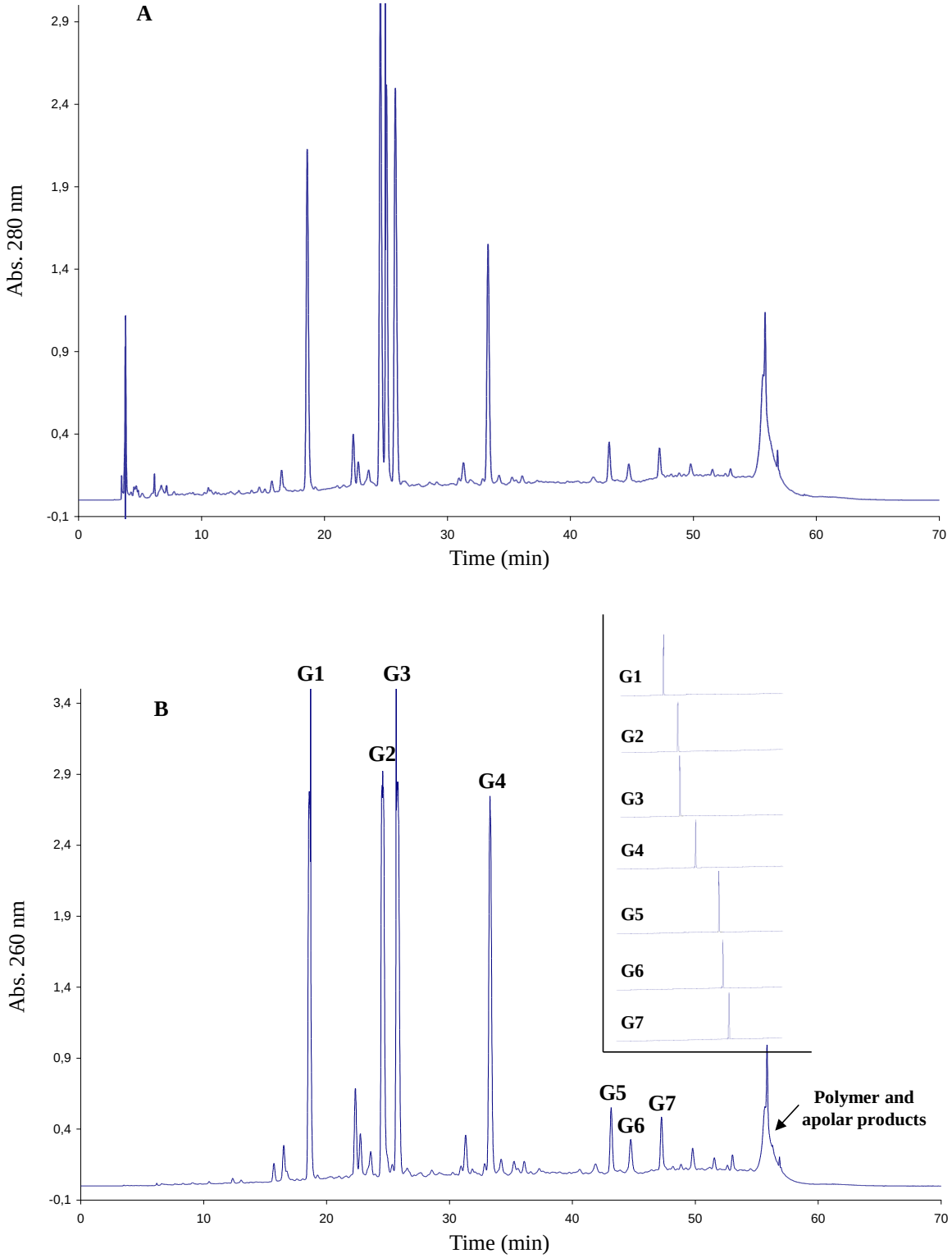
Isolation of melanoidin fraction in the case of coffee: yield = 2, 5 g from 10 g of powder (25% of dry matter) and in the case of toasted oak wood : yield = 0,5 g from 10 g of powder (5% of dry matter).

We noted first the difference of yield in the two cases. Coffee matrix was richer in melanoidins than toasted oak wood, it is the most abundant substrate, the percentage of melanoidins was more important because coffee is more roasted than toasted oak wood.

We developed an analytical HPLC method in order to analyze the total fraction composition from coffee and toasted oak wood. HPLC/DAD technique (Figures 1 and 2) permits us to well separate several peaks which present the same spectrum UV-Visible with a maximum of absorbance at 260 nm, specific of melanoidin spectrum. Each peak was collected by semi-preparative HPLC. In the case of roasted coffee we obtained seven fractions: G1, G2, G3, G4, G5, G6, G7, see Figure 1. At the end of the HPLC chromatogram, the peak of polymers and apolar substances is low. In the case of toasted oak wood, we obtained four fractions: F1, F2, F3, F4, see Figure 2. The peak of polymers and apolar substances is more important. HPLC chromatograms show the excellent purity of each product collected nearly 100% (Figures 1 and 2, Table 1).

Table 1. Proportions of each purified fraction from coffee and toasted oak wood melanoidin total fractions

Coffee melanoidin fraction		***Toasted oak wood melanoidin fraction***	
Fractions	**Weight (mg)**	**Fractions**	**Weight (mg)**
Fractions obtained by semi-preparative HPLC		**Fractions obtained by semi-preparative HPLC**	
G1	2	F1	1.2
G2	1.8	F2	1.9
G3	2.2	F3	2.3
G4	1.9	F4	1.4
G5	0.6	**Fractions obtained by low pressure chromatography**	
G6	0.4	P1	6
G7	0.5	P2	7.5
		P3	5
		P4	13
		P5	15
		P6	11
		P7	10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Abs. 280 nm
2,9
2,4
1,9
1,4
0,9
0,4
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0
10
20
30
40
50
60
70
Time (min)
B
Abs. 260 nm
3,4
2,9
2,4
1,9
1,4
0,9
0,4
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G1
G2
G3
G4
G5
G6
G7
Polymer and
apolar products
Time (min)

Figure 1.

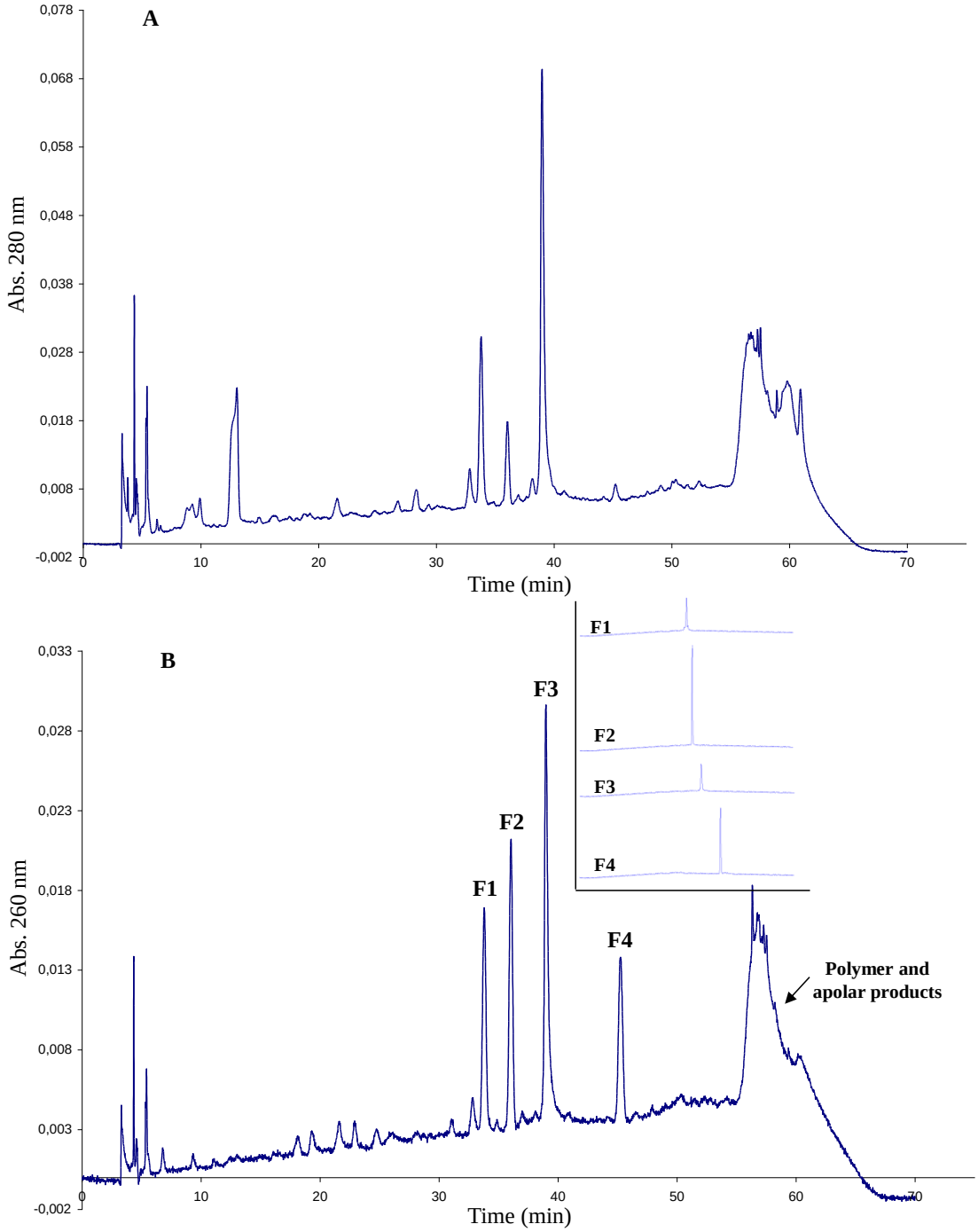


Figure 2.

In the case of toasted oak wood, we developed a second method using low pressure gel chromatography to obtain higher quantitatively product and to separate the polymer form. The different fractions, obtained and detected by UV detector at 280 nm, were collected manually: P1, P2, P3, P4, P5, P6, P7 were the obtained fractions (Figure 3, Table 1). HPLC chromatograms show the excellent purity of each product collected nearly 100%. We retained the second method in order to obtain pure fractions from TFMW, we separated thanks to this technique the polymer form no fractionated by HPLC column.

The fractions P were analyzed by different analytical techniques: LC/ESI-MS, IR and NMR.

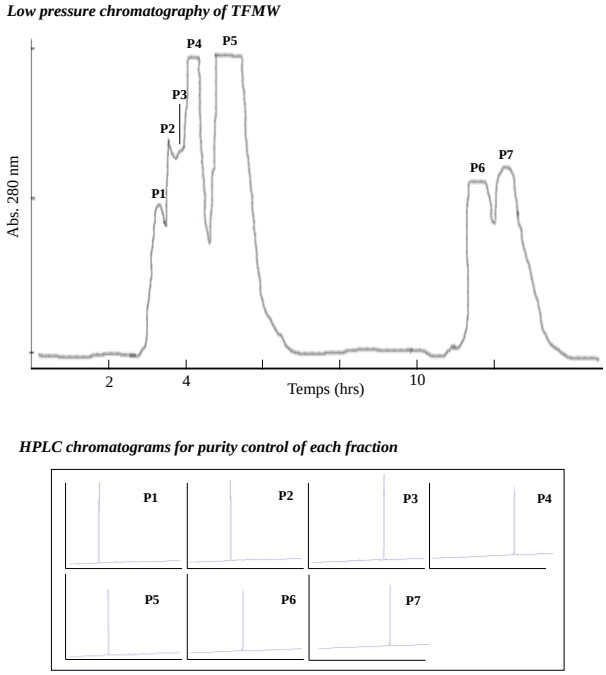


Figure 3.

3.2 Thermal Degradation Studies

The total fraction of melanodins (TFMC and TFMW) obtained respectively from coffee and toasted oak wood were heated at 250 ℃ (10 min) and the produced volatiles were analyzed by SPME-GC-MS (Figures 4 and 5). To this purpose, firstly we subjected the isolated melanoidins from oak wood to thermal destruction at 250 ℃ in an inert atmosphere. The especially selected high temperature was to ensure a sufficient degree of conversion of the melanoidin molecules and to provide us with better information relating to their ability to generate volatile products.

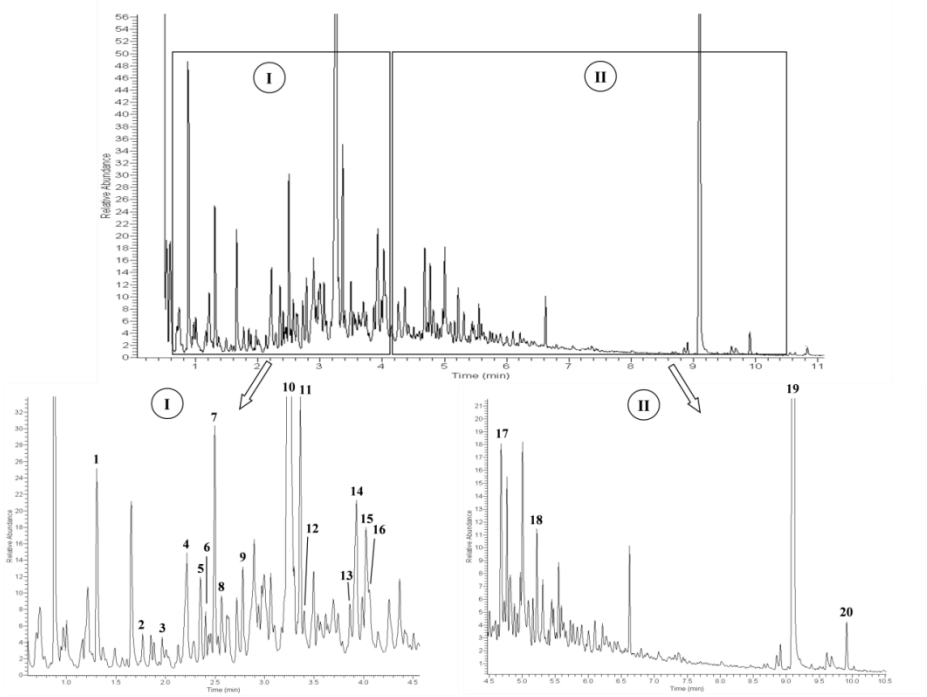


Figure 4.

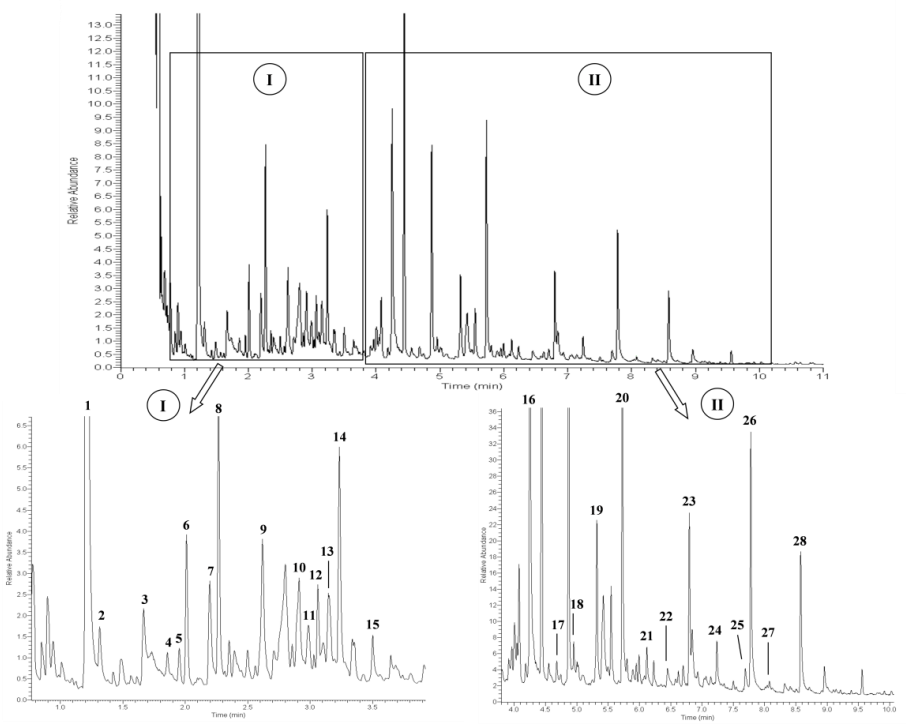


Figure 5.

In the case of coffee, the data are given in Table 2; as can be seen the mixture contains 20 components. The furan group is represented in eight compounds, the total amount being 60%. The highest content is that of 2(3H)-furanone, 5-acetyldihydro-, and 5-hydroxymethyldihydrofuran-2-one. Alicyclic derivatives were found (12%) as well as maltol, see Table 2.

Table 2. Data for the thermal degradation products of melanoidins from coffee

N °	Tr (min)	Molecular peak	Compound
1	1.30	98	2-Furanmethanol
2	1.77	106	Pyrazine, ethenyl-
3	1.85	107	Pyridine, 3-ethyl
4	2.22	102	3-Hydroxydihydro-2(3H)-furanone
5	2.35	95	1H-Pyrrole-2-carboxaldehyde
6	2.41	120	Pyrazine, 2-ethenyl-6-methyl
7	2.50	112	2-Cyclopenten-1-one, 2-hydroxy-3-methyl
8	2.57	130	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl,
9	2.78	109	Ethanone, 1-(1H-pyrrol-2-yl)-
10	3.23	126	Maltol
11	3.36	128	2(3H)-Furanone, 5-acetyldihydro-
12	3.40	109	3-Pyridinol, 2-methyl
13	3.87	153	Imidazole, 2-amino-5-[(2-carboxy)vinyl]
14	3.93	116	5-Hydroxymethyldihydrofuran-2-one
15	4.02	110	Pyrocatechol
16	4.06	133	Pyridine,2-methyl-5-(1-methylethenyl)-
17	4.68	142	2(3H)-Furanone, 3-acetyldihydro-3-methyl
18	5.22	156	2(3H)-Furanone, dihydro-5-pentyl
19	5.32	194	Caffeine
20	9.91	210	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)

The headspace profil of the oak wood melanoidins (Figure 5) consisted mainly of furans (74% of the total GC peak area), a small amount of pyrroles and pyrazines, see Table 3. Furfural, maltol, and isomaltol, which are important compounds in the headspace of heated oak wood melanoidins, are typical caramelization products of sugars.

Table 3. Data for the thermal degradation products of melanoidins from toasted oak wood

N °	Tr (min)	Molecular peak	Compound
1	1.22	96	Furfural
2	1.31	98	2-Furanmethanol
3	1.67	84	Cyclopentanone
4	1.86	112	2,5-Furandione, 3-methyl
5	1.95	139	1H-Imidazole-4-ethanamine, N, 5-dimethyl-
6	2.01	110	5-Methyl furfural
7	2.20	102	3-Hydroxydihydro-2(3H)-furanone
8	2.27	143	Oxazolidine, 2,2-diethyl-3-methyl
9	2.62	153	Imidazole, 2-amino-5-[(2-carboxy) vinyl]
10	2.91	112	2-Furancarboxylic acid
11	2.99	111	2-Furanmethanamine, N-methyl
12	3.06	169	Pyrrolidizine-3-one, ethyl ether
13	3.15	271	(+)-S-Phenethanamine, 1-methyl-N-vanillyl
14	3.23	126	Maltol
15	3.50	144	4H-Pyran-4_one, 2,3-dihydro-3,5-dihydroxy-6-methyl
16	4.25	126	5-(Hydroxymethyl) furfural
17	4.68	152	2,6-Dihydroxyacetophenone
18	4.95	168	2-Furancarboxaldehyde, 5-[(acetyloxy)methyl]
19	5.32	154	Phenol 2,6-dimethoxy
20	5.73	152	Vanillin
21	6.12	223	2,5-Dimethoxy-4-ethylamphetamine
22	6.23	166	Phenol, 2-methoxy-4-propyl
23	6.80	180	2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)
24	7.24	141	Pyrrolidine, 2-butyl-1-methyl
25	7.69	156	N-Methyl-3-hyrdoxymethylpyrrolidin-2-one
26	7.78	182	Benzaldehyde, 4-hydroxy-3,5-dimethoxy
27	8.08	194	Phenol, 2,6-dimethoxy-4-(2-propenyl)-
28	8.58	210	2-Pentanone, 1-(2,4,6-trihydroxyphenyl)

In Figures 6 and 7, some representative structures of compounds identified in the headspace of heated melanoidins respectively from TFMC and TFMW are depicted.

From the results presented it can be seen that during manufacturing process destruction of melanoidins is likely to take place, producing volatile components which take part in the formation of the flavour complex in the finished product.

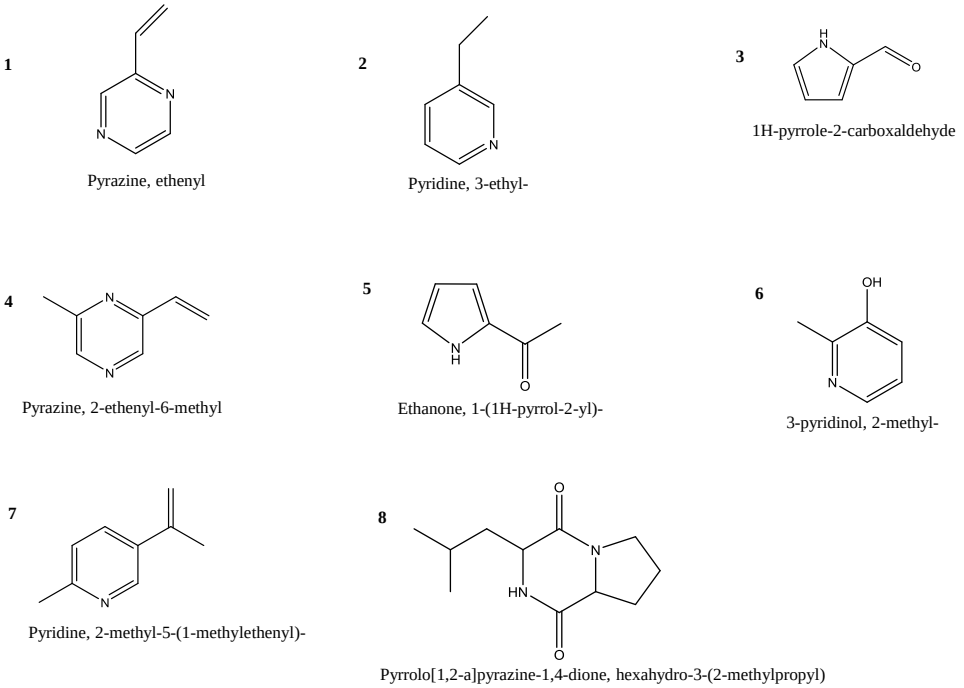


Figure 6.

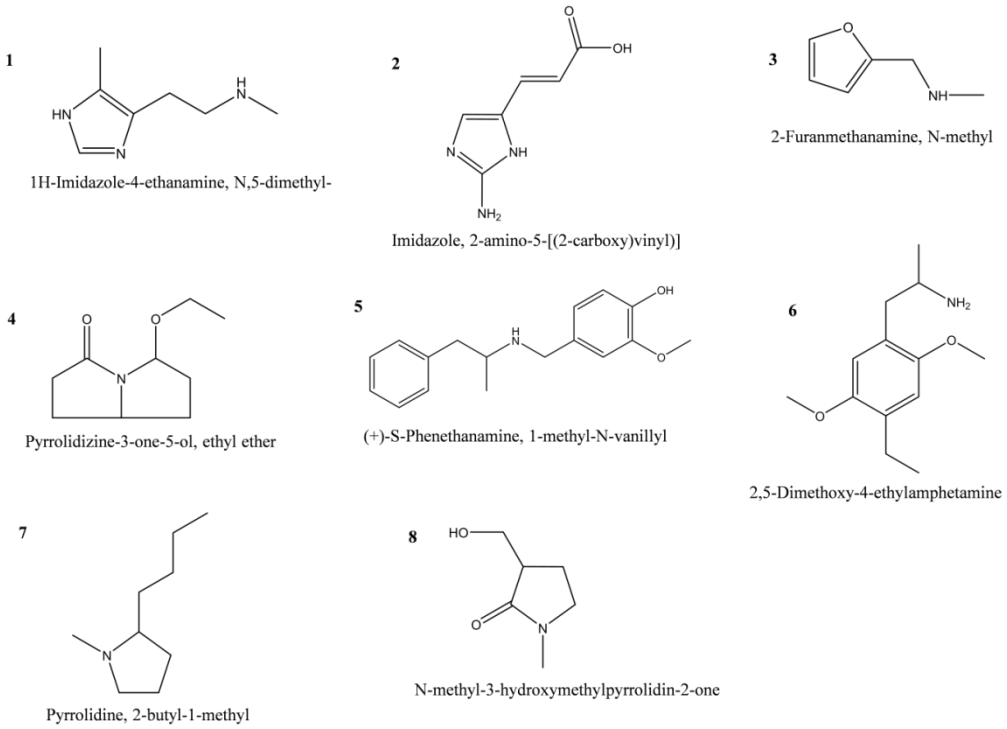


Figure 7.

3.3 Characteristics of the Melanoidins Isolated from TFM

3.3.1 Elemental Analysis (C, H, O, N) and Viscosimetry

The chemical investigations in our study revealed that coffee and toasted oak wood melanodins have different elemental (CHON) compositions and similar viscosity values, see Table 4. Coffee melanoidin fraction (TFMC) contain 2,65% of nitrogen, 42,15% of carbon, 5,10% hydrogen, and 50,10% of oxygen. Toasted oak wood melanoidin fraction (TFMW) contain 1,95% of nitrogen, 39,95% of carbon, 6,86% hydrogen, and 50,96% of oxygen, and the formula for their ratio is $C_{24}H_{49}O_{23}N$. These data are similar to those obtained for the quantitative element composition of known model and food melanodins (Cämmerer & Kroh, 1995; Margarita et al., 1996). The percentage of nitrogen is more important in the case of coffee fraction; it was easily explain by the fact that coffee contained a large majority of melanoidins.

Table 4. Elemental compositions of coffee melanodin fraction and toasted oak wood melanoidin fraction

Coffee melanoidin fraction (TFMC)				
Component Name	**Average**	**Std. Deviation**	**% Relative S.D.**	**Variance**
Nitrogen	2.65	0.1089904	4.1385	0.0119
Carbon	42.15	0.9364315	2.2227	0.8769
Hydrogen	5.1	0.03153798	0.6288	0.001
Oxygen	50.1	0.9804534	1.336	0.9134
Toasted oak wood melanoidin fraction (TFMW)				
Component Name	**Average**	**Std. Deviation**	**% Relative S.D.**	**Variance**
Nitrogen	1.95	0.0802004	3.04531	0.0087
Carbon	39.95	0.8875548	2.1067	0.8311
Hydrogen	6.86	0.04242167	0.8457	0.001
Oxygen	50.96	0.9867877	1.465	0.9078

Viscosity of each fraction is around 1,466 mPa.s in the case of oak wood and 1,545 mPa.s in the case of coffee. The viscosity in each case varied according to the temperature (Table 5). More temperature increased and more viscosity decreased and inversely.

Table 5. Viscosty measurements from coffee and toasted oak wood fractions

Coffee melanoidin fraction (TFMC)			
Fractions	**Viscosity at 15 ℃ (mPa.s)**	**Viscosity at 20 ℃ (mPa.s)**	**Viscosity at 25 ℃ (mPa.s)**
G1	1.631	1.545	1.52
G2	1.632	1.547	1.521
G3	1.632	1.545	1.525
G4	1.632	1.544	1.524
G5	1.631	1.546	1.525
G6	1.631	1.545	1.526
G7	1.635	1.544	1.52
Toasted oak wood melanoidin fraction (TFMW)			
Fractions	**Viscosity at 15 ℃ (mPa.s)**	**Viscosity at 20 ℃ (mPa.s)**	**Viscosity at 25 ℃ (mPa.s)**
P1	1.51	1.466	1.435
P2	1.511	1.467	1.434
P3	1.515	1.467	1.433
P4	1.511	1.465	1.435
P5	1.511	1.465	1.433
P6	1.51	1.466	1.434
P7	1.51	1.466	1.433

3.3.2 UV-Visible and IR Spectra

The UV and IR spectra are characteristics of melanoidins (Figures 8, 9 and 10). The absorption maximum at about 260 nm is broad; this is characteristic of polymer compounds containing a large number of chromophores which a different nature.

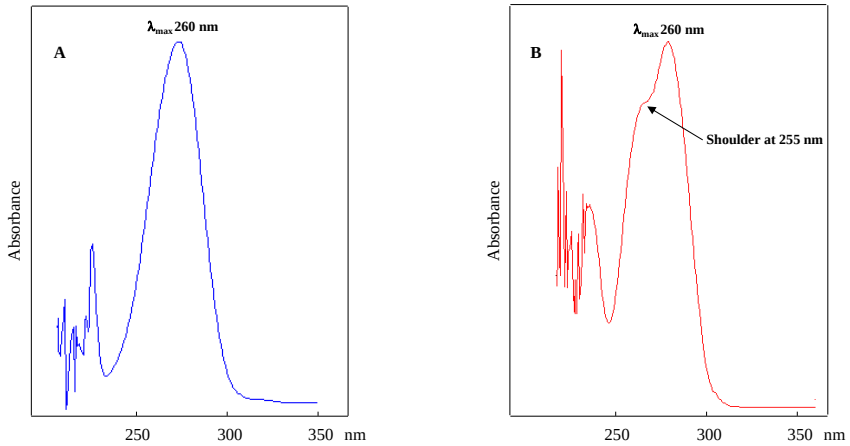


Figure 8.

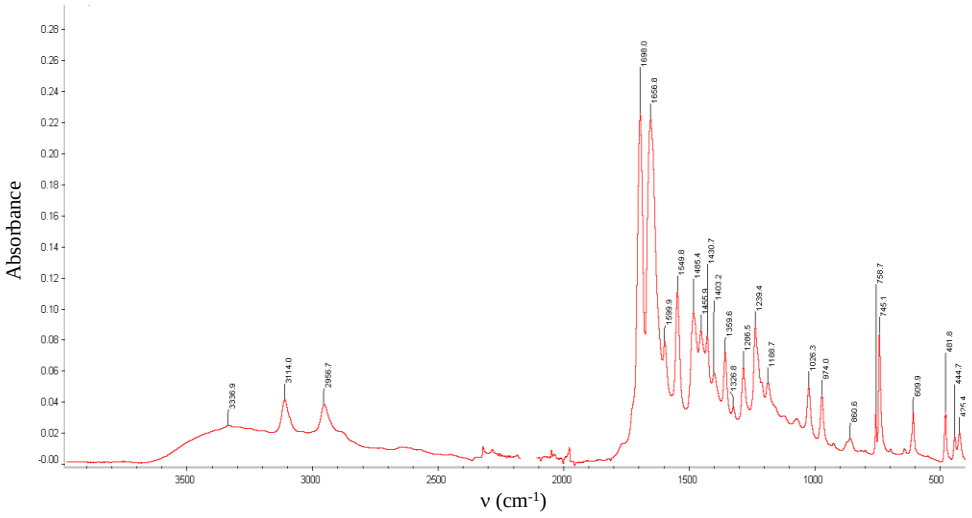


Figure 9.

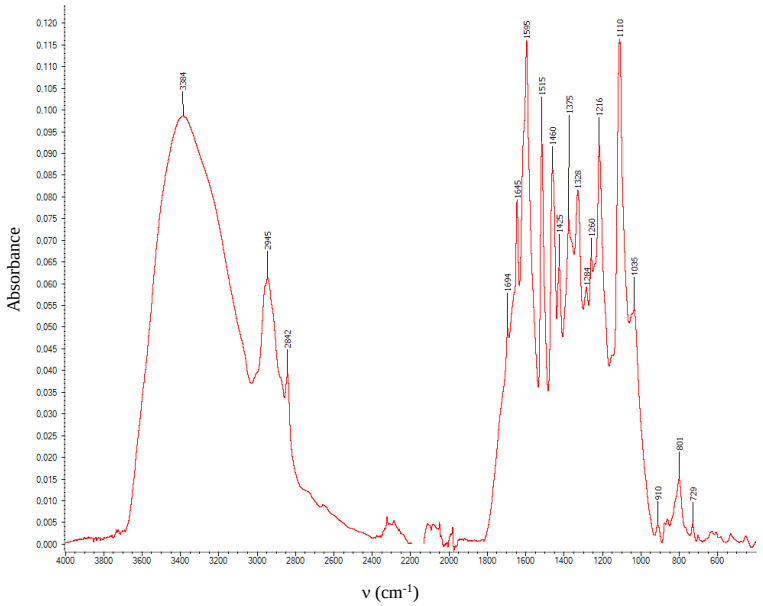


Figure 10.

The IR spectra (Figure 10) of the different fractions : P1, P2, P3, P4, P5, P6, P7 are similar in the case of toasted oak wood, and they are typical of polymeric compounds with spreading bands. In the same way the IR spectra (Figure 9) of the different fractions: G1, G2, G3, G4, G5, G6, G7 in the case of coffee are similar and present the same characteristic bands.

Nevertheless some characteristic absorptions are observed which allow assignment to group frequencies. Characteristic frequencies in the case of oak wood (Table 6) are observed for C-OH groups at 1035 cm^{-1}, C=O (-COOH) groups at 1700 cm^{-1}, C=N and C=C groups at 1644 cm^{-1}. The band at 2946 cm^{-1} can be attributed to the hydrogen stretching vibrations in CH_2 and CH_3 groups, the broad band at 3382 cm^{-1} to the –O-H and –N-H stretching. The same bands are observed in the case of coffee, see Table 7.

Absorptions are in agreement with the general hypothetical melanoidin structure presented in different studies (C ämmerer & Kroh, 1995; Poirier et al., 2000; Rubinsztain et al., 1986; Rubinsztain et al., 1986).

Table 6. Assigments of the bands in infrared spectra of toasted oak wood melanoidin fraction

Frequencies, n (cm^{-1})	Intensities		Assignment
3382	vs		n O-H, n N-H
3100-3500	mw		polymeric H-bonding (O-H)
2946	s	Strongly coupled modes	n_{AS} C-H (CH_3, CH_2)
2842	m		n_S C-H (CH_2, O-CH_3)
1644	m		n C=C, n C=N, n C=O
1596	vs		n C=C aromatic
1516	s		n C=C (para substitued aromatic)
1461	s		d CH_2 aliphatic
1426	m		d CH_2 aliphatic
1375	m		d -CH_3, d -C-O-H (phenol)
1329	m		n_{AS} C-O-C
1217	s		n C-O-H (phenol), n C-N
1112	vs		d >C-O-H (secondary alcohol), d C-O-CH_3, n C-N aliphatic
1035	m		n C-O, n C-N, n C-C skeletal
802	m		d_{op} aromatic, d C=C-H alcanes

w, wide; m, medium; ms, medium strong; s, strong; vs, very strong
ν: stretching
ν_S: symetric stretching
ν_{AS}: asymetric stretching
δ: bending
δ_{op}: bending out of plane

Table 7. Assigments of the bands in infrared spectra of coffee melanoidin fraction

Frequencies, n (cm^{-1})	Intensities	Assignment
3337	w	n O-H
3100-3500	s	polymeric H-bonding (O-H)
3114	ms	n_{AS} CH_3
2957	ms	n_S CH_3
1698	vs	n C=O
1658	vs	n C=O, n C=C
1600	s	n C=C, n C=N
1550	s	n N-CH_3, d ring, CH_3 rock
1485	s	d_S CH_3, n CN, d CH_3
1456	s	d_S CH_3
1431	s	d_S CH_3, n CN, d CH
1403	ms	n C-N in imidazole ring
1360	s	n_S NCH_3 + n CN + d CH_3
1287	ms	n NCH_3, n C-N & n C-C in rings
1239	s	d C-H, g CH_3 rock, n C-N
1189	ms	d C-H bend, n CN, r CH_3
1026	ms	CH_3 rock (in plane), n C-N
974	ms	d_{ip} pyrimidine ring, r CH_3
745	vs	n N-CH_3, d_{ip} imidazole ring, t CH_3
610	s	d_{op} imidazole ring (t NCN)
482	ms	d_{ip} pyrimidine ring, d CNC

w, wide; m, medium; ms, medium strong; s, strong; vs, very strong
ν: stretching
ν_S: symetric stretching
ν_{AS}: asymetric stretching
δ_S: symetric bending
δ_{ip}: bending in plane
δ_{op}: bending out of plane
ω: wagging
τ: torsion
r: rocking

3.3.3 LC/ESI/MS Analysis

Carbohydrates are the most important substrates in the Maillard reaction and melanoidin formation. Both, monosaccharide degradation products and intact sugars play a role in the formation of the melanoidin backone (ref). Therefore, to obtain further insight into the carbohydrate composition of the melanoidins, the isolated melanoidins fractions (P) from TFMW low pressure gel chromatography were analysed using LC/ESI/MS.

Mass spectrum of Fraction P1, obtained by electrospray ionisation in the negative ion mode, was obtained (Figure 11).

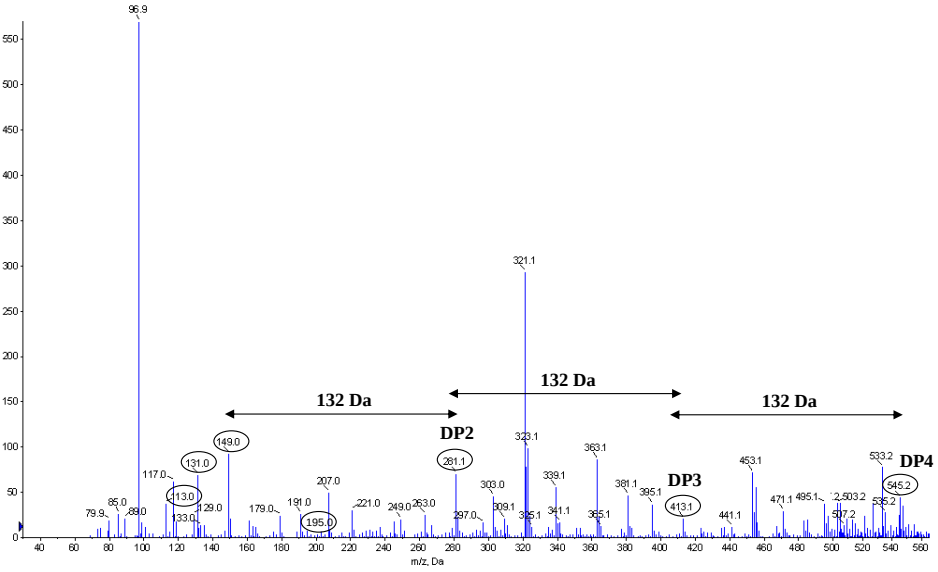


Figure 11.

The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 545, m/z 413, m/z 281, m/z 149 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses. The fragmentation patterns of monosaccharides, disaccharides, trisaccharides and oligosaccharides have been studied by Verardo, Duse, and Callae and Brudzynski et al. using LC/ESI tandem mass spectrometry negative ion mode. The examination of mass spectra in negative ESI/MS^n of the fragmentation patterns of pentoses by those authors produced a list of fragmentation ions identical to those found in the mass spectrum of the Fraction P1.

Mass spectrum of Fraction P2, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 545, m/z 413, 281, 149 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectrum of Fraction P3, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 789, 657, 525, 393, 261 and 129 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 261) to DP5 (m/z 789). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectrum of Fraction P4, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 373, 241, 109 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 241) to DP3 (m/z 373). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses. Mass spectrum showed fragment ions, specifically m/z 89, 113, 143, 161, and 179, that are characteristic of the fragmentation pattern of hexoses.

Mass spectrum of Fraction P5, obtained by electrospray ionisation in the negative ion mode, was obtained (Figure 12).

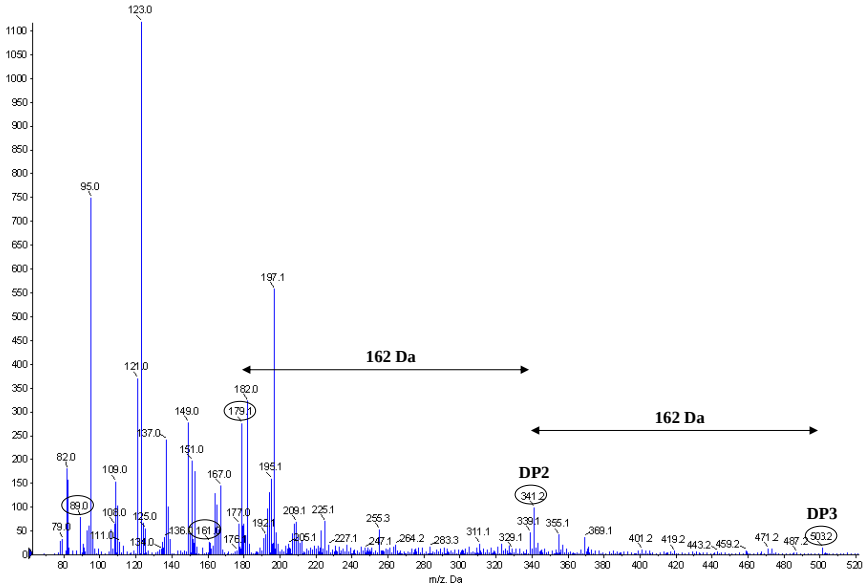


Figure 12.

The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 503, 341, 179 formed a series differing by 162 amu. The 162 amu corresponds to anhydrous hexose and therefore the above ions

indicate the degree of hexose polymerisation (DP) from DP2 (m/z 341) to DP3 (m/z 503). Mass spectrum showed fragment ions, specifically m/z 89, 113, 143, 161, and 179, that are characteristic of the fragmentation pattern of hexoses.

Mass spectrum of Fraction P6, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 581, 317 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation: DP2.

Mass spectrum of Fraction P7, obtained by electrospray ionisation in the negative ion mode, was obtained. The spectrum was dominated different peaks which, with the individual [M-H$^+$] ion at m/z 551, 419 formed a series differing by 132 amu. The 132 amu corresponds to anhydrous pentose and therefore the above ions indicate the degree of pentose polymerisation (DP) from DP2 (m/z 281) to DP4 (m/z 545). Mass spectrum showed fragment ions, specifically m/z 113, 131, 149, and 195, that are characteristic of the fragmentation pattern of pentoses.

Mass spectra of the fractions isolated from TFMW indicate the presence of pentose and hexose-based oligosaccharides with different degrees of polymerisation. The presence of the oligosaccharide moieties, as well as their degradation products found in the oak wood melanoidins, supports the postulated carbohydrate-based origin of melanoidins.

In the case of TFMC, the fractions G were analysed using LC/ESI/MS. Mass spectra of fractions G1, G2, G3 were presented respectively on Figures 13, 14, 15. Fractions G3, G4, G5, G6 and G7 presented the same mass spectrum.

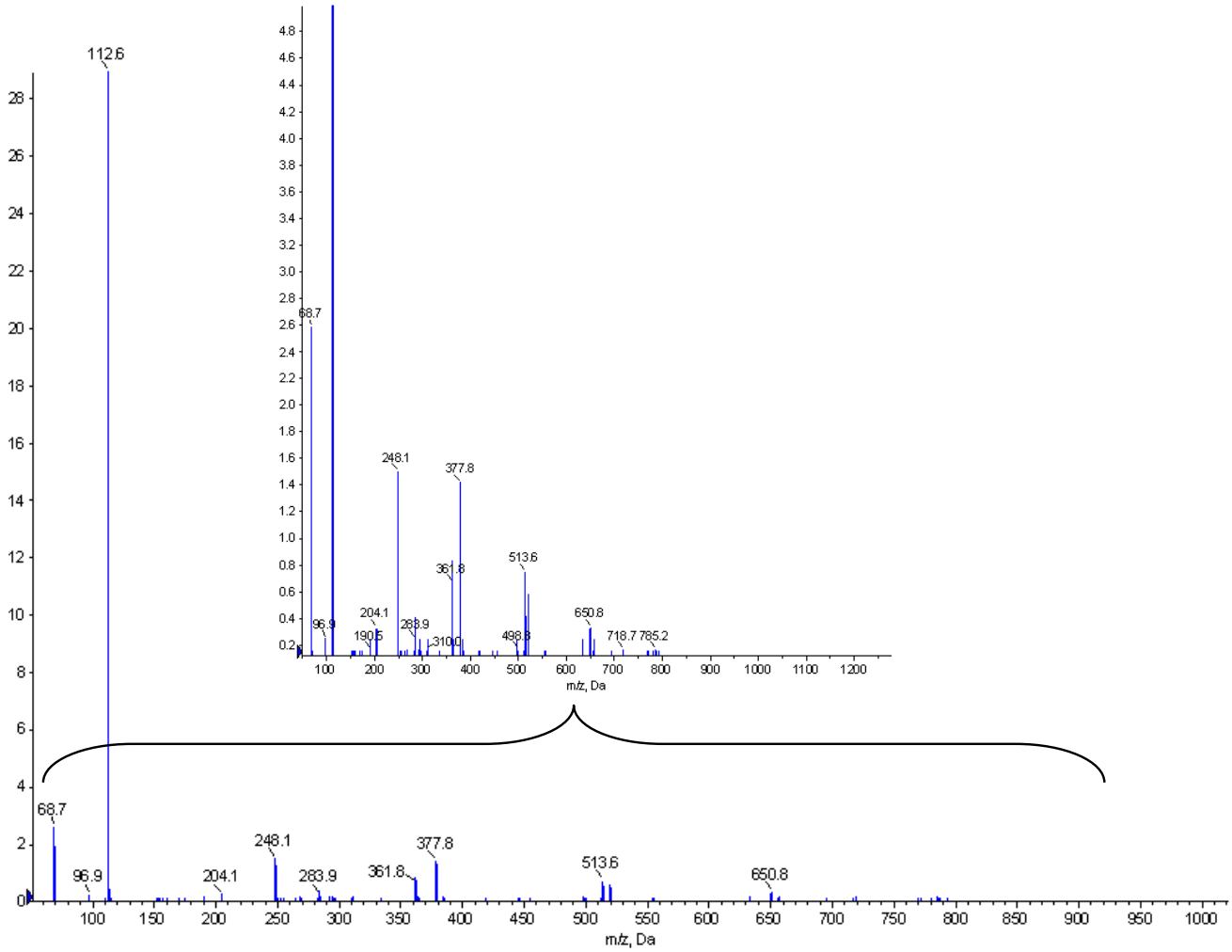


Figure 13.

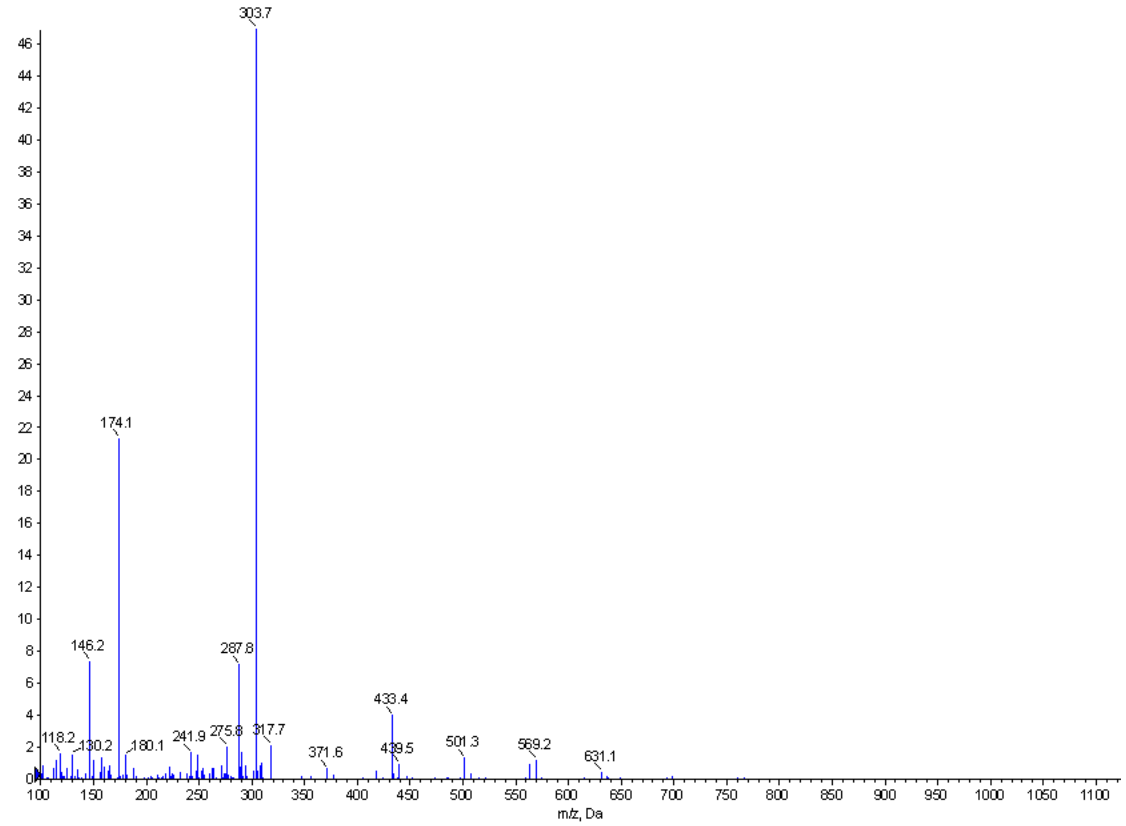


Figure 14.

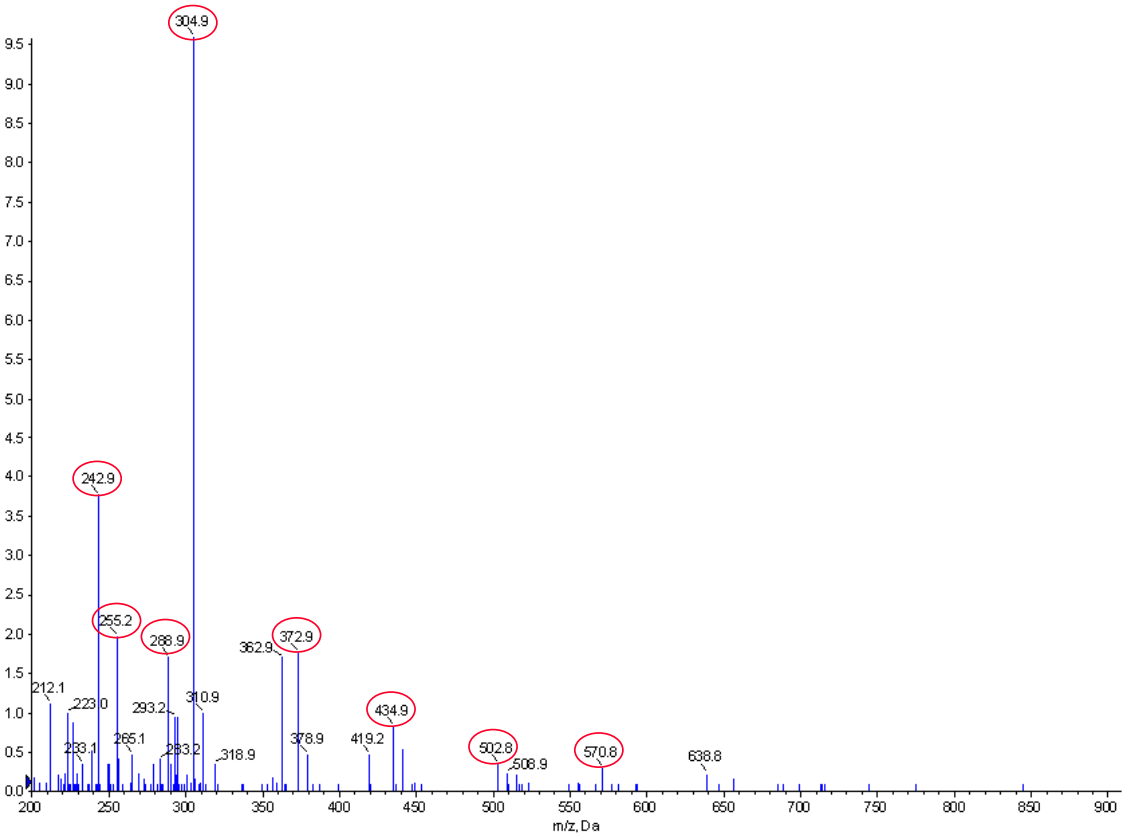


Figure 15.

3.4 Structure of Melanoidin Polymer

Melanoidins are high molecular weight amino-carbonyl compounds. In general, the separation of melanoidins from food and other biological samples is very difficult and therefore, most of the chemical and biological studies about melanoidins have done on model melanoidins. Although the chemical structure of melanoidins is not understood clearly, some part of the chemical structure of model melanodins have been elucidated by NMR techniques (Allard et al., 1997; Fang & Schmidt-Rohr, 2009; Gniechwitz et al., 2008).

Figure 16 presented ^{1}H NMR spectra of each P purified fractions from toasted oak wood.

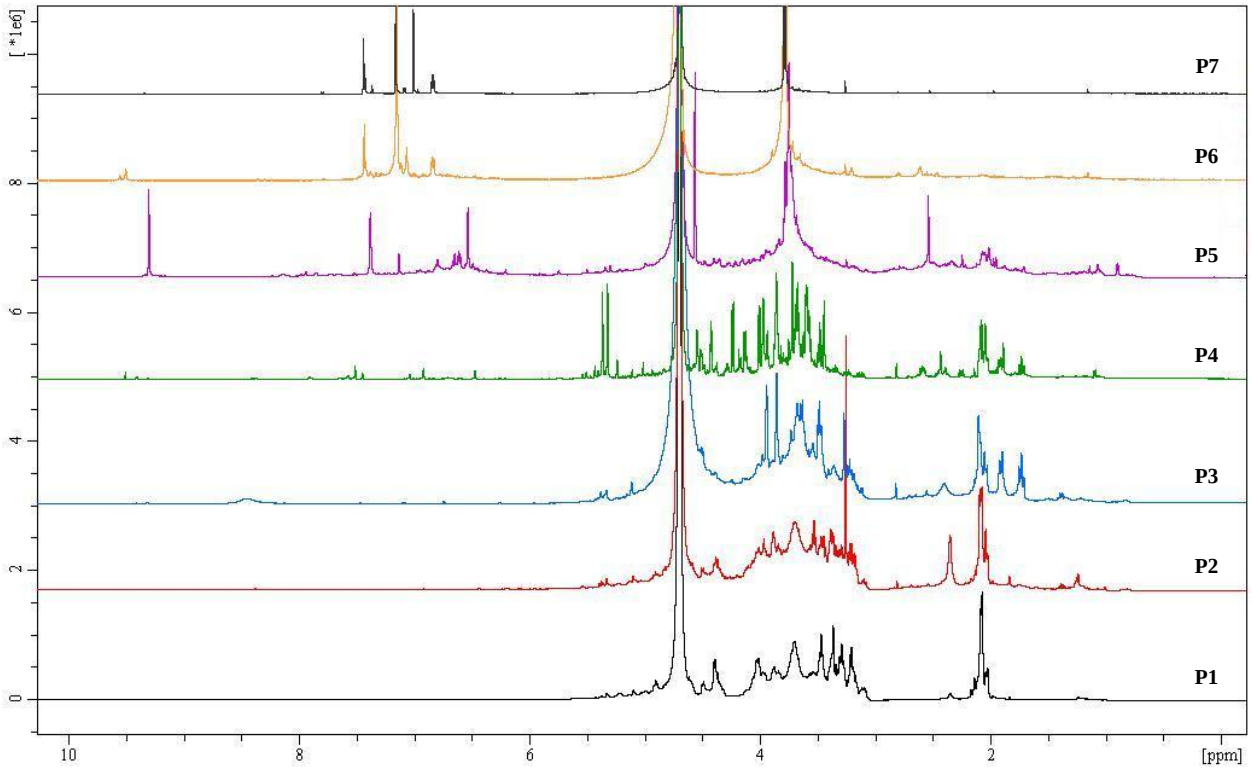
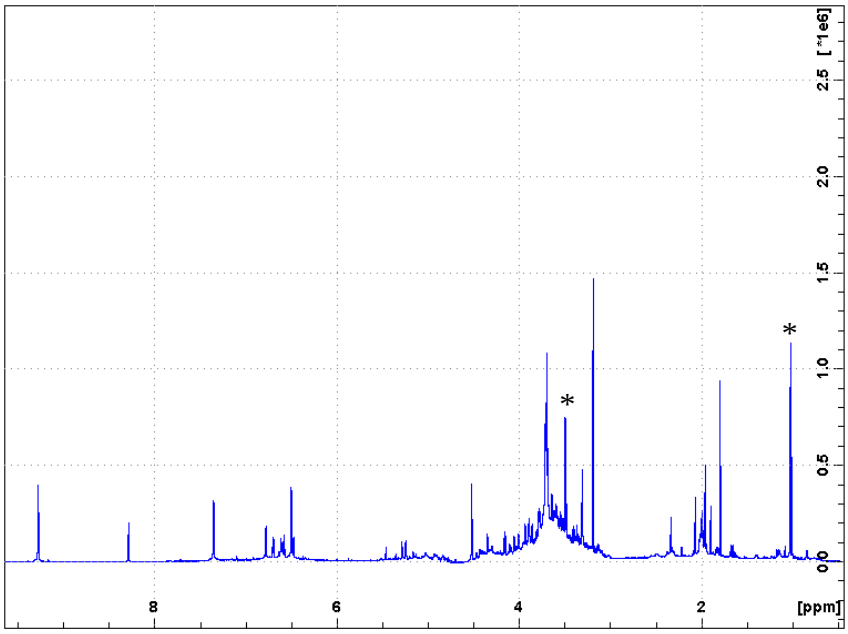


Figure 16.

Figures 17 and 18 presented respectively ^{1}H NMR and ^{13}C spectra of P4 fraction with experimental data. ^{1}H - ^{13}C correlations were obtained with normal 2D techniques HSQC and HMBC (Table 8).

Table 8. Assignments of the ^{1}H and ^{13}C chemical shifts in NMR spectra of toasted oak wood melanoidin fraction

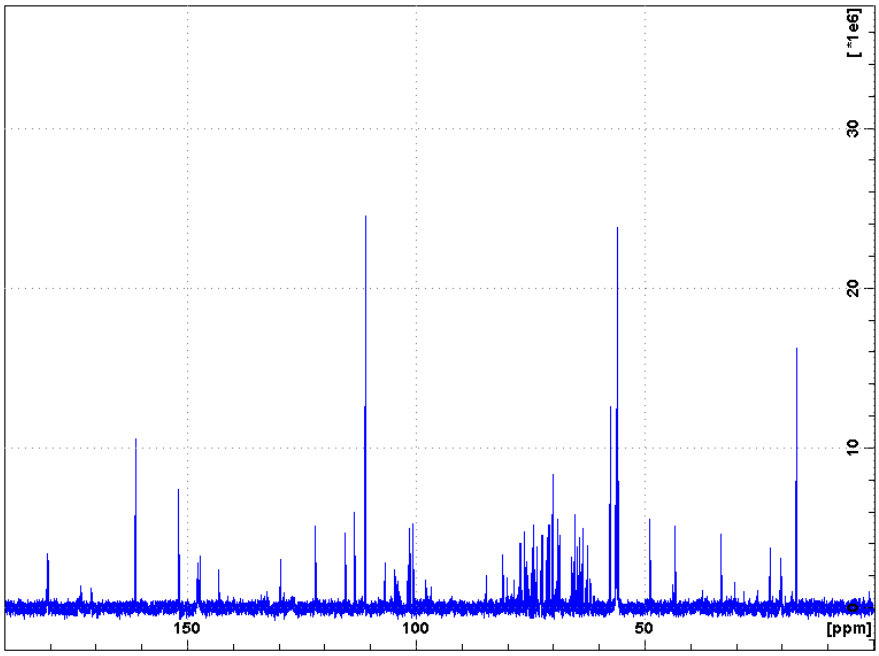
	^{1}H (δ, ppm)	^{13}C (δ, ppm)	Groups or functions
1	9.28	180.4	Aldehyde >C=O
2	8.29	_	
3	_	162<δ<150	Heteroaromatic >C=N
4	6.0<δ<7.5	101.6<δ<122	Alkene, aromatic >C=C<
5	5.0<δ<5.5	91.9<δ<104.5	Anomeric proton and carbon
6	3.0<δ<5.0	62.4<δ<101.5	Sugar ring
7	0.5<δ<2.5	17.6<δ<37.4	



Experimental data

^{1}H NMR (D_2O, 800.23 MHz) δ 9.28 (s, 1H), 8.29, 7.35 (d, 1H, J = 3 Hz), 6.78 (s, 1H), 6.70 (d, 0.5 H, J = 5.5 Hz), 6.64 (br, 0.2 H), 6.61 (t, J = 5.3 Hz), 6.58 (s), 6.57 (s), 6.50 (d, J = 3.1 Hz), 6.48 (br), 5.46 (s), 5.35-5.0 (complex signals, 1H), 4.52 (s), 4.50-3.0 (complex signals, 40 H), 2.34 (s), 2.28-1.55 (complex signals, 6H), 1.50-1.09 (complex signals, 3H)

Figure 17.



Experimental data

^{13}C NMR (D_2O, 201.22 MHz) δ 180.4, 161.2, 151.7, 147.5, 147.1, 143.1, 129.5, 121.8, 115.3, 113.3, 110.9, 106.6, 104.5, 104.3, 104.1, 103.8, 103.7, 103.5, 101.6, 101.3, 101.1, 100.5, 97.8, 96.5, 84.4, 80.9, 79.9, 78.5, 77.6, 77.1, 76.1, 75.6, 75.4, 74.7, 74.5, 74.4, 74.1, 73.4, 72.6, 72.3, 72.1, 71.3, 71.1, 70.8, 70.6, 70.0, 69.9, 69.1, 68.8, 68.5, 65.8, 65.1, 64.5, 64.1, 63.4, 62.3, 61.8, 57.3, 56.1, 55.9, 55.7, 48.7, 43.7, 43.3, 33.1, 22.5, 20.1, 16.7.

Figure 18.

These correlations permitted to observe and confirm some groups and functions: aldehyde >C=O, heteroaromatic >C=N-, alkene, aromatic >C=C<, anomeric proton and carbon, and sugar rings. These results were in correlation with those obtained in IR and ESI/MS.

The determination of the exact chemical structure of these compounds will be achieved by using other classical battery of 1D and 2D NMR structural experiments.

4. Discussion

We studied the extractible forms of melanoidins produced by toasting oak heartwood and coffee beans. For these two different sources of melanoidins, maillard reactions represent an important transformation of the raw material, improving the quality of products and develop a large part of these aromatic interest and diversity. Because coffee melanoidins are well documented, we used it as a model to develop our specific method on the toasted oak heartwood melanoidins.

We noted that the quantities of melanoidins in coffee and oak wood are very different. Coffee is richer in melanoidin compounds than oak wood. This is probably due to the roasting step that is different in the two cases. The roasting process, in the case of coffee, is more favorable to the production of melanoidins than the toasting process in the case of oak wood. The roasting temperatures and the toasting temperatures are comparable but the times in each case is different.

Specialists situate “roasting zone” between 185 and 240°C, the optimum being between 210 and 230 ℃. Above of these temperatures starts over-roasting. The time of the roasting is usually 12 to 15 minutes. The temperature roasting and its conduct have considerable influence on the qualities of coffee.

In the case of oak wood, we noted the presence of a larger amount of melanoidin polymers probably in relation with the heating time (± 50-55 min). The time of roasting coffee is around 15 to 20 min in the case of traditional roasting. This may explain the lower extraction because more the degree of polymerization increase and more the extractability decrease. So in oak wood, the extractible melanoidins are less present than in the case of coffee; this may also explain the difference in content melanoidins in both matrices.

In addition of the total amount of melanoidins, the polymeric status of both melanoidins are different.

After the maillard reaction, inducing the formation of melanoidins, the thermic treatment permitted the degradation of melanoidins, source of various aroma : vanilla for vanillin, almond for furfural, toasted almond for 5-methyl furfural, toasted for maltol.

The Pyrolysis GC of coffee melanoidins product less of various compounds, about 20, against 28 for Pyrolysis/GC of oak wood melanoidins. It exist probably a relation between degree of polymerization and number of molecules product and identify by pyrolysis technique.

Pyrolysis is the decomposition of an organic compound by a significant increase in its temperature (450 ℃) to obtain other products. At this high temperature, polymerized melanoidins, more present in oak wood melanoidins, are decomposed and produce more simple organic compounds.

From measurements of the viscosity of the solution for different concentrations of dissolved polymer, one can calculate the viscosity-average molecular weight (ref).

The viscosity of the melanoidins oak wood (1,46 ±0.08) is less than that of coffee melanoidins (1,54 ±0.08), while oak wood present higher molecular weight melanoidins.

Oak wood melanoidins contain less nitrogen (1,9%) than coffee melanoidins (2,6%). Green coffee contain from 1 to 3% total nitrogen, engaged in various combinations, the main ones are proteins and alkaloids (ref). Amino acids in general and certain amino acids containing sulfur (cystine and methionine especially), contained in the coffee proteins play an important role in the formation of the roasted coffee aroma. Among the amino acids that make up green coffee proteins, some of them, such as arginine, cysteine, lysine and serine, showed a high decrease in their amount during roasting (ref review). Oak wood contain less than 1% total nitrogen. And toasting process have also an impact on the degradation compounds.

We noted that the absorption maximum is about 260 nm, characteristic of nucleic acid absorbance. Nucleic acids are macromolecules with nitrogen groups, that is to say relatively large complex molecules. They enter in the family biomolecules as they are of great importance in the kingdom of life.

The nucleic acids consist of a chain of nucleotides linked by phosphodiester bonds. Nucleotides always consist of two basic components: a sugar (ose with 5 carbons or pentose) and nucleic base or nitrogenous base. This corroborates the observations made by LC/ESI/MS technique. Mass spectra of the different fractions in the

case of oak wood, show the presence of pentoses linked with another element. We proposed a hypothetic structure with a degree of pentose polymerization, DP=5.

The IR spectra present some characteristic frequencies and bands similar in the case of oak wood melanoidins and coffee melanoidins : C-OH groups, C=O (-COOH) groups, C=N and C=C groups, band corresponding to the hydrogen stretching in CH_2 and CH_3 groups, band corresponding to the –O-H and –N-H stretching.

In the case of coffee melanoidins, fractions didn't present frequencies at 800 cm^{-1} (aromatic bending) and 1110 cm^{-1} (C-N aliphatic stretching, C-O-CH_3 bending), but presented supplementary frequencies at : 745 (N-CH_3 stretching), 974 (bending pyrimidine ring), 1287 (N-CH_3, C-N stretching), 1485 (CH_3 symetric bending, C-N stretching), 1698 cm^{-1} (C=O stretching), with strong intensities.

Acknowledgment

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