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Electron spin resonance and electron nuclear double resonance spectroscopy study of non-dialysable melanoidin pyrolysates

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ABSTRACT

Free radicals with extended lifetimes at room temperature, formed by pyrolysis (from 473 to 773 K) of the non-dialysable melanoidins, were studied by electron spin resonance (ESR) and electron nuclear double resonance (ENDOR) spectroscopy. The ESR spectra showed an identical symmetrical absorption line with $g = 2.0035 \pm 0.0009$ and with line-shape changing depending on the carbonisation temperatures. The ENDOR spectra of the free radicals possessed similar characteristics in all the cases and did not show significant couplings of the unpaired electron on a C atom to α-protons ($A_{iso} = 14.35$ MHz). This comparison of ESR and ENDOR results provides insight into paramagnetic electron Heisenberg spin exchange interaction existing in melanoidin molecules.

Non-dialysable melanoidin; ESR and ENDOR spectroscopy

INTRODUCTION

Trials for establishing the structure of non-dialysable melanoidins, as the end-reaction product of the Maillard reaction between aldoses and amino acids, are essential for further elucidation of the temperature effects on the reaction pathway of melanoidin formation in the heated alkaline solution. It is well known that browning of the aldose–amino acid model systems increases with temperature [1,2] and time [3,4]. The complexity of the carbonyl–amine reaction, as seen from recently reported results [5], makes it almost impossible to study it by means of simple analytical or organic chemical methods, but the electron spin resonance (ESR) and electron nuclear double resonance (ENDOR) spectroscopy approaches can be used in attacking this problem and they seem to be reasonable.

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EXPERIMENTAL

The chemicals used in the present investigations were of analytical purity. Model systems and melanoidin preparation have been described in the previously published work by Milić [6]. The starting materials of non-dialysable melanoidins were purified several times from deuterium oxide to dilute out the light protons and then analysed.

The ESR examinations were carried out as described in the recently published work by Milić and Piletić [2].

The ENDOR studies were performed on the Bruker 300 Spectrometer in the high temperature X-band cavity ER 4114 HT, with the addition loop of wire to produce the intense magnetic field perpendicular to the static magnetic field, $\mathbf{H}_0$, and the microwave magnetic field, $\mathbf{H}_1$.

The *g*-tensors for the carbonium-centered species were calculated with a non-linear least-squares method. Raw data for the *g*-factors were evaluated from ESR spectra, with the magnetic field values taken from the spectrometer, and the klystron frequency monitored by a Hewlett–Packard model 5245L frequency counter with 5257A transfer oscillator. Final calibration of the *g*-factors was performed by comparing the g_{max} peak of the species powder spectrum with a DPPH marker. The coupling *g*-tensors from ENDOR data were derived by using a two-step technique of trial tensor generation by Schonland's method [7] followed by a non-linear least-squares fit to the actual ENDOR data [8].

RESULTS AND DISCUSSION

Electron spin resonance (ESR) and electron nuclear double resonance (ENDOR) studies of pyrolysation series consisting of samples of non-dialysable melanoidins were carried out as a part of a continuing investigation of the chemistry and structure of melanoidins obtained from heating model systems of D(+)-glucose and aminobutyric acid isomers.

The melanoidin samples pyrolysed at temperatures below 373 K revealed no observable ESR signals. All other pyrolysed samples showed an identical symmetrical absorption line with $g = 2.0035 \pm 0.0009$ (Fig. 1).

Lineshapes of the ESR spectra curves obtained in the temperature range from 373 to 673 K are of lorentzian type in the middle, while the wings are of gaussian shape. With increasing carbonisation temperature the deviation of the lineshape from the shape of the lorentzian curve decreased. For the pyrolyses at a temperature below 373 K, it was impossible to investigate the lineshape in detail because of the lower intensity of ESR signal. The behaviour of the lineshape in the temperature treatment range between 373 K and 673 K can be explained by the exchange interaction between the electron spins in identical organic radicals, which causes the transition to

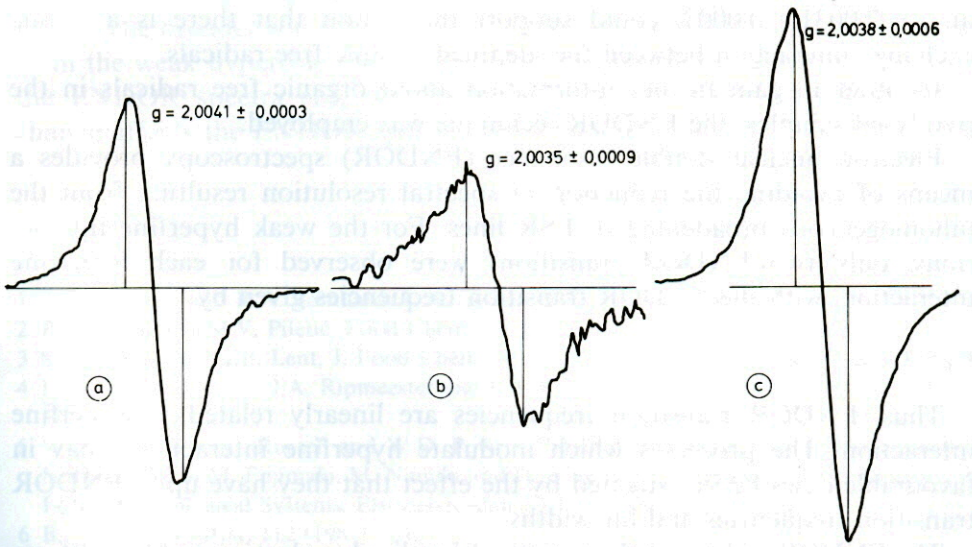

Fig. 1. ESR spectra of non-dialysable melanoidin pyrolysates obtained at 673 K from the D(+)-glucose and 2-, 3- and 4-aminobutyric acid model systems (a, b, c).

have a nearly lorentzian shape. The estimation from the linewidth, H_{p-p} indicated that the frequency of this interaction was about 10^7 s^{-1}, comparing that with a diphenylpicrylhydrazyl, DPPH, single crystal value of 10^{11} s^{-1}, as a well known stable organic free radical system.

All studied samples showed saturation phenomena for the maximum microwave power at the resonator at 10 mW. The evaluation of the spin-lattice relaxation time, T_1, from the saturation behaviour yields for the samples pyrolysed at 673 K, was $T_1 = 10^{-6}$ s. The saturation broadening of the samples pyrolysed between 373 K and 673 K was inhomogeneous, indicating that the linewidth, H_{p-p}, in this temperature range is mainly determined by the unresolved hyperfine structure of the protons.

The spin concentrations of samples changed dramatically within the given temperature range. Up to 573 K, only moderate changes in spin concentration occurred. Spin concentrations increased sharply at about 673 K and dropped substantially at 773 K.

In order to characterise organic free radicals in the samples pyrolysed at 673 K the signal intensity of the ESR spectra was measured as a function of temperature in the range from 115 to 273 K and the results were compared with those from DPPH and the Bruker pitch. For the temperature range employed, the signal intensity of the samples increased with decreasing temperature at the rate predicted by the Curie–Weiss law. The systems that follow this law often have a paramagnetic doublet ground state independent of their surroundings. The absence of hyperfine structures, relatively narrow linewidth independent of temperature, as well as quasi-lorentzian lineshape

at $g = 2.0031 \pm 0.0009$, could support the notion that there is a strong exchange interaction between the identical organic free radicals.

In order to gain further information about organic free radicals in the pyrolysed samples, the ENDOR technique was employed.

Electron nuclear double resonance (ENDOR) spectroscopy provides a means of avoiding the reduction in spectral resolution resulting from the inhomogeneous broadening of ESR lines. For the weak hyperfine interactions, only two ENDOR transitions were observed for each hyperfine interaction, with the ENDOR transition frequencies given by

$$\nu_{\pm} = \nu_N \pm A/2$$

Thus, ENDOR transition frequencies are linearly related to hyperfine interaction. The processes which modulate hyperfine interactions may in favourable cases be investigated by the effect that they have upon ENDOR transition frequencies and linewidths.

The ENDOR studies of the variety of localised π-electron radical systems demonstrate that resolved spectra are often obtained and that these can be interpreted by appropriate theory. A similar observation holds for delocalized π-electron radicals when hyperfine interactions are not modulated by defect diffusion or by Heisenberg spin exchanges. The single-peak signal of ENDOR spectra (Fig. 2) at the free proton frequency, thus providing the direct evidence, showed that environments of free radicals in the pyrolysed samples contained hydrogen atoms which had interaction with the unpaired electron on a C atom. Since the ENDOR signals from the three pyrolysed samples at 673 K were almost identical, this indicates that the environment of the unpaired electrons in each sample is essentially the same.

In general, ENDOR spectra of all examined melanoidins were observed to consist of components corresponding to two or more different structures of hydrocarbons. The narrow component, which possesed the unsaturated

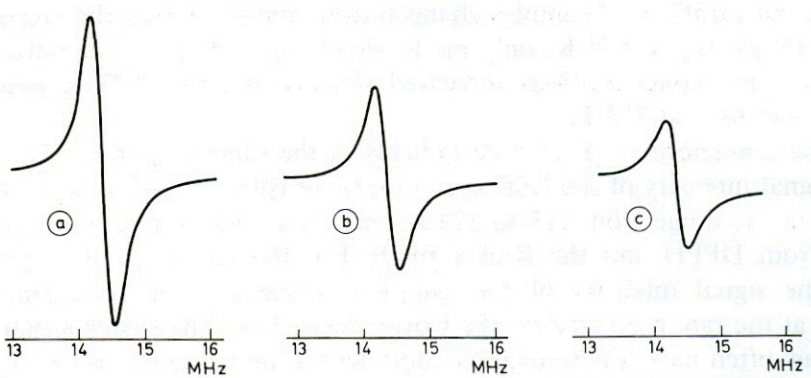

Fig. 2. ENDOR spectra of non-dialysable melanoidin pyrolysates obtained at 673 K from the D(+)-glucose and 2-, 3- and 4-aminobutyric acid model systems (a, b, c).

structure, at the free proton Larmor frequency might be a distant ENDOR signal. The broader component, which possesses aromaticity, probably arises from the weak hyperfine interactions. The comparison of these results from the ENDOR spectra (Fig. 2) suggests that electron Heisenberg spin exchange affects the ENDOR spectra of melanoidins.

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