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Starch radicals. Part I. Thermolysis of plain starch

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Plain starch from two varieties of potato and wheat, one variety of rye, triticale, oat, corn, waxy corn, cassava and amaranthus was thermolyzed subsequently at 170, 250, 285, 300 and 325°C for 30 min–2 h intervals. The concentration of unpaired spins was determined. Among varieties studied, the oat starch is least thermally resistant, and both the triticale and maize starches are most thermally resistant. Radicals generated in such a manner are very stable. The analysis of experimental and simulated EPR spectra points to the unpaired spin delocalization and steric hindrances are responsible for the stability of such radicals. © 1997 Published by Elsevier Science Ltd. All rights reserved.

INTRODUCTION

There is a certain concern about radicals, thermally generated from carbohydrates among others, in thermal food-processing. Radicals are readily formed on the heating of mono- and disaccharides. Hence, for instance, caramels contain a remarkable concentration of unpaired spins. The radicals are unusually stable (Abagyan & Apresyan, 1979). There were several theories explaining their stability in ammonia caramels. The aza-heterocyclic components of secondary food aromas are considered to be systems providing perfect unpaired spin delocalization (Hashiwagi & Enomoto, 1981). However, as shown by Barabasz *et al.* (Barabasz *et al.*, 1990) plain caramels from mono- and di-saccharides might also contain a high unpaired spin concentration. Nevertheless, such caramel showed no mutagenicity in tests with *Escherichia coli*. Radicals generated from D-fructose, D-glucose and sucrose showed a limited reactivity in attempting to use them in crosslinking starch and cellulose (Tomasik & Jane, 1995).

In addition, starch develops radicals when either heated to give British gums or subjected to ionizing radiation (Tomasik *et al.*, 1989). Unpaired spins were unevenly distributed between crystalline and amorphous regions of starch granules (Raffi & Agnel, 1983). As suggested by Sultankhodzhayeva *et al.* (1983) as well as by Thiery *et al.* (1990) the radicals resulted from the homolytic cleavage of the C—H, C—OH and CO—H bonds. Although such radicals may decompose by dehydration in a- and b-eliminations and a cleavage of the C—C and C—O bonds in the D-glucosyl residues of starch matrix, such radicals, again, are very stable and survive almost annual air exposure (Tomasik & Jane, 1995).

In this paper the resistance of a variety of starch to radical cleavage is presented and an explanation of the extraordinary stability of the radicals is attempted.

MATERIALS AND METHODS

Materials

Potato starch was manufactured by Niechlow Potato Enterprise in 1993 (the first variety, PD) and by PPH Matizolbud Zalesice, Poland, in 1995 (the second variety, PM). Cassava starch (C), maize (M), waxy maize (WM) and amaranthus (A) starch was kindly provided by the Central Laboratory of the Potato Industry in Poznan (Poland). All other starch varieties were isolated from available sources in the laboratories of the Department of Carbohydrate Technology of the University of Agriculture in Cracow [Dankowskie zlote rye (R), Dargo triticale (T), Emika wheat (WE), commercial oat (O) and commercial wheat (WH) starch].

Methods

Thermolysis
Samples of starch (0·1 g) were thermolyzed in porcelain crucibles using an ELF 11/6 Eurotherm Carbolite oven with an analogous digital temperature stabilizer provid-

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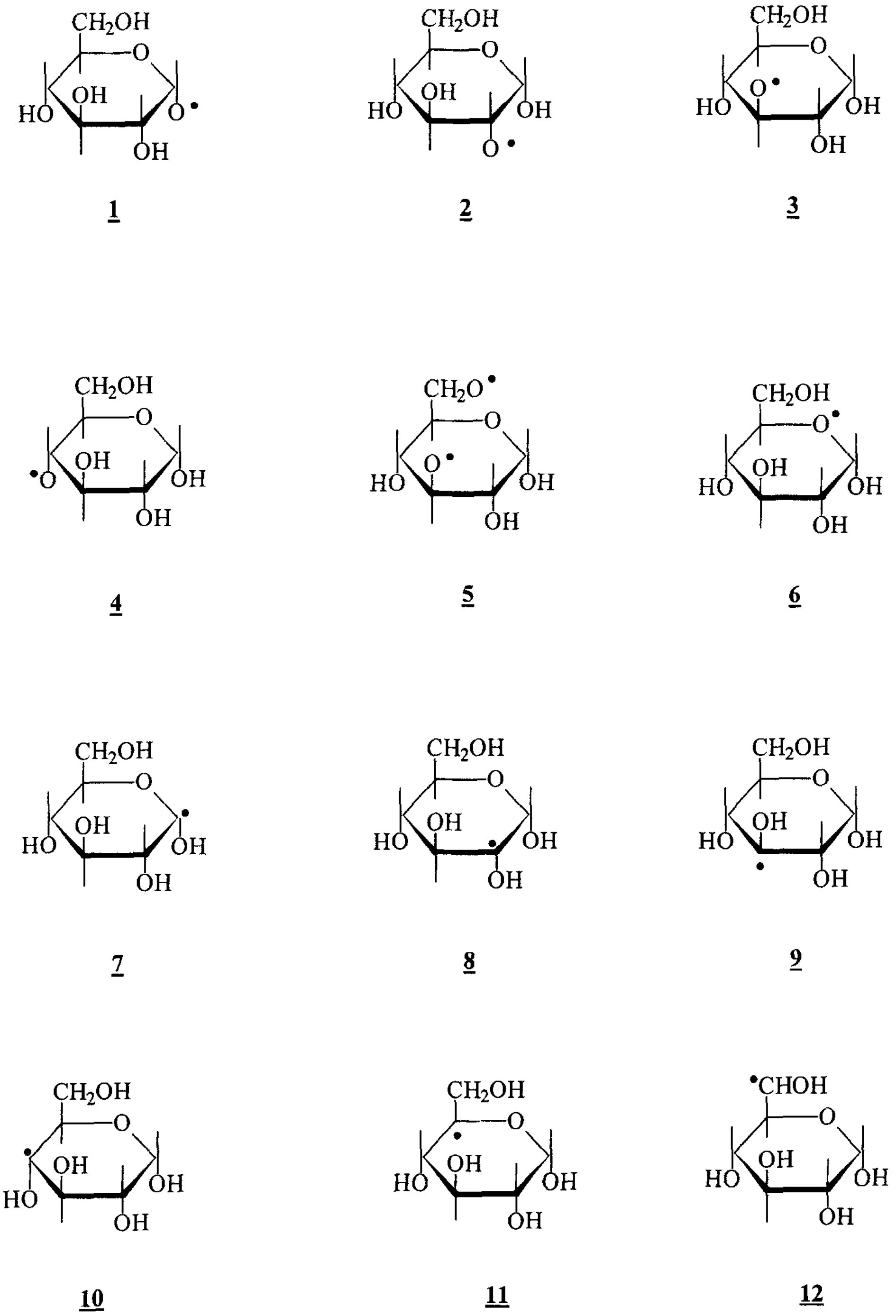
ing ±0·5°C heating precision. The samples were heated to 170, 250, 285, 300 and 325°C for 30 60, 90 and 120 min in all posible parameter combinations. Thermolyzed samples were subjected to further studies either directly after the thermolysis or after extraction. The extraction was carried out either with water (the sample was either left in water at room temperature for 24 h or heated on a steam bath for 30 min) or with ethanol (24 h in a Soxhlet apparatus). Air dried samples of starch as well as dry residues from evaporated extracts were investigated by means an EPR spectrometer. The reaction with iodine was run solely for extracted, thermolyzed samples of oat, potato (Niechlow), waxy corn and corn starch.

EPR spectra

EPR spectra were recorded for powdered samples in the X-band region (n @ 9·5 GHz, l = 3·2 cm) at room temperature. $CuSO_4$ was taken as the standard for spin abundance ($2{\cdot}4\times10^{21}$ spins/g) and DPPH was the standard for the g-factor. The apparatus was manufactured by Politechnika Wroclawska (Poland). The spectral



1 2 3

4 5 6

7 8 9

10 11 12

Fig. 1. Structures of glucosyl radicals for which the EPR spectra were simulated.

curves were processed using the 2·8 b MicroCal Origin program.

Thermolyzed starch–iodine complexes

The complexes were prepared from thermolyzed, extracted samples (0·01 g) by heating in water (5 cm^3) followed by the addition of iodine solution in KI (2 cm^3). This solution was prepared by blending iodine (2·538 g) with 1×10^{-1} mol/dm^3 of aqueous KI (0·1 dm^3) followed by 1:10, 1:200 and 1:500 dilutions. The spectra were recorded with UV-VIS Specord apparatus in 10 mm quartz cells in the range of 200–700 nm. The 625 nm-band shift and absorbances at 625 and 545 nm were taken for our consideration, as these corresponded to the absorption maxima for iodine complexes with amylose and amylopectin (Baldwin *et al.*, 1944).

EPR spectra simulation

The simulation of the EPR spectra for 12 glucose radicals bearing one unpaired spin, localized subsequently at either an oxygen or a carbon atom (Fig. 1), was carried out under the assumptions listed in Table 1 with either intermolecular spin–spin interactions ($B_0=3000$ G, $\Delta B=1000$ G) or in their absence ($B_0=3000$ G, $\Delta B=70$–120 G). The program NIEHS-LMB-EPR from the EPR Software Program (1992) for running Brooker and Varian spectrometers was utilized in the simulation.

RESULTS

Table 2 presents the unpaired spin counts in thermolyzed starch samples, in respect of $CuSO_4$ as the standard. The g-factor for all samples at n @ 9·5 GHz calculated according to Equation 1

$$g = h\nu/\mu B \tag{1}$$

is approximately 2·003 and very close to the g-value for the signal for DPPH.

Table 1. Parameters accepted in the simulation of the EPR spectra of glucosyl radicals[a]

Structure number[b]	Splitting constants[c], G	Structure number[b]	Splitting constants[c], G
1	25 (H_1) 4, 1 5 (H_2) 1 2 (H_3) 1 1 (H_5) 1	7	25 (H_1) 4, 1 5 (H_2) 1 2 (H_3) 1 1 (H_4) 1
2	25 (H_1) 4, 1 5 ($H_{2,\ 3}$) 2 2 (H_4) 1 1 (H_5) 1	8	25 ($H_{1,\ 2}$) 4, 4 5 (H_3) 1 1 (H_4) 1 2 (H_4) 1
3	25 (H_1) 3, 1 5 ($H_{2,\ 3}$) 2 2 ($H_{4,\ 5}$) 2	9	25 ($H_{1,\ 2}$) 3, 2 5 ($H_{3,\ 4}$) 2 2 (H_5) 2
4	25 (H_1) 4, 1 5 ($H_{2,\ 3}$) 2 2 (H_4) 2 2 (H_5) 1	10	25 ($H_{1,\ 2}$) 4, 2 5 (H_3) 2 5 (H_4) 1 2 (H_5) 1
5	25 (H_1) 7, 2 5 (H_2) 1 2 (H_3) 1 1 (H_4)1 25 (H_3) 1 5 ($H_{2,\ 5}$) 2	11	25 (H_1) 5, 2 25 (H_2) 1 1 (H_5) 1 5 (H_3) 1 (H_4) 1 2 ($H_{4,\ 6}$) 2
6	5 ($H_{1,\ 2}$) 5, 2 5 (H_5) 2 5 (H_3)1 5 (H_4) 1 2 (H_6) 1	12	20 (H_1) 5, 1 25 (H_2) 1 5 (H_3) 1 1 (H_4) 1 2 (H_5) 1

[a] The nuclear spin $s=0.5$ was accepted for all calculations.
[b] The structure number corresponds to numbering in Fig. 1.
[c] The splitting constants are followed by the number of splitting constants (the same for all calculations within the given structure) and the number of equivalent nuclei.

Visually the thermolyzed samples turned gradually from white to brown, dark brown and black. Only very dark, practically black, samples contained unpaired spins as proved by the EPR signals. Contrary to other starch samples, the sample of oat starch showed about 40% weight loss on 90 min heating at 285°C, and it lost nearly 80% of its original weight on 120 min heating at 325°C.

The treatment of thermolyzed starch with cold water

Table 2. Number of spins, *n*, in starch of various origin[a], $n\times10^{15}/g$[b] roasted at 285–325°C

Temperature (°C)	Time (min)	$n\times10^{15}/g$										
		PM	PN	T	O	WE	WC	R	M	C	WM	A
285	90	—	—	—	10	—	—	—	—	—	—	—
	120	—	—	5	20	6	4	8	1	—	2	—
300	90	5	7	2	10	2	6	2	—	6	3	5
	20	6	10	3	30	3	5	4	3	7	2	5
325	60	—	—	—	4	—	—	—	—	—	—	—
	90	40	10	4	80	3	70	4	2	10	4	12
	120	200	80	8	1000	40	600	60	7	50	10	40

[a] Starch of the following origin: PM—potato (Matizolbud); PN—potato (Niechlow); T—triticale; O—oat; WE—wheat (Emica); WC—wheat commercial; R—rye; M—maize; C—cassava; WM—waxy maize; A—amaranthus.
[b] $CuSO_4$ was taken as the standard.

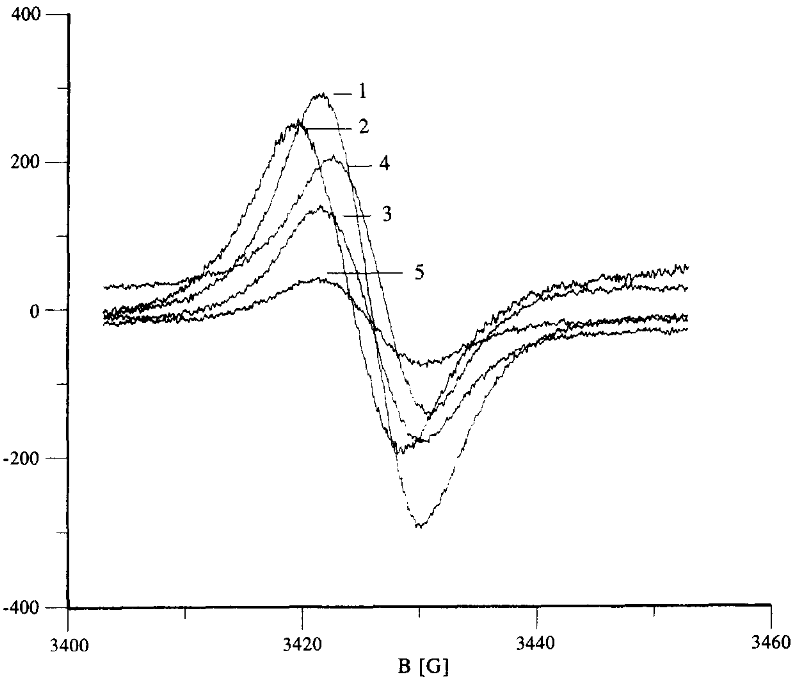


Fig. 2. The experimental EPR spectra of potato starch thermolyzed at 300°C for 2 h prior to extraction (14.1×10^{-3}*g*) (1); treated with cold water followed by vacuum evaporation to dryness without filtration (13.8×10^{-3}*g*) (2); after the extraction with hot water (8.1×10^{-3}*g*) (3); after the extraction with cold water (14.3×10^{-3}*g*) (4); after the extraction with ethanol (15.3×10^{-3}*g*) (5).

resulted in the reduction by half of the free radical count in the solid residue. The samples of thermolyzed starch lost approximately 75% of unpaired spins after extraction with hot water, and approximately 85% of unpaired spins after ethanolic extraction.

Figure 2 presents the EPR spectrum of potato starch prior to extraction (sample weight = 0·0141 g) (1); after the treatment with water followed by vacuum evaporation to dryness without filtration (sample weight = 0·0138 g) (2); after hot water extraction (sample weight = 0·0081 g) (3); after cold water extraction (sample weight = 0·0143 g) (4); and after ethanolic extraction (sample weight = 0·0153 g) (5).

The temperature-dependent extention of the destruction for four starch varieties is presented in Fig. 3. These data illustrate the variation of the absorbances (A) of the complex of iodine with the thermolysis products recorded at 625 and 545 nm (Tomasik *et al.*, 1996). This variation is presented as the A_{625}/A_{545} ratio in the spectra measured for the 1:200 dilution. Figure 4 presents the EPR spectrum for all structures (1–12 in Fig. 1) simulated with intermolecular spin–spin interaction and for structures 1 and 5, respectively, when intermolecular spin–spin interactions were assumed to be absent.

DISCUSSION

One may see that oat starch was the least thermally stable and triticale, cassava and maize starches were the most stable, in terms of the number of generated spins. This stability was not related to the amylose to amylopectin ratio, because the number of spins in dextrins, resulting from waxy maize starch being almost pure amylopectin, should take a terminal position in the order of either decreasing or increasing spin count. Admixtures such as protein and/or lipids in the form of native starch complexes could have an essential influence on the thermal stability of starch. Proteins and partially lipids as thermally unstable, readily decomposed and decomposition products promoted generation of radicals from starch. It might be deduced from a significant weight-loss of the oat starch on heating. The loss reached 40% within 90 min of heating at 285°C and almost 80% after 120 min of heating at 325°C. This behavior of oat starch was unique, because other starch varieties did not loose weight on such heating. The high thermal stability of maize starch might result from lipids present in it as the component of helical amylose complexes. The lipids present in oat starch reside, partly in the form of sorption complexes, and can be extracted from starch, and partly in nonextractable helical complexes (Gibiński *et al.*, 1993). Distinctly more thermally stable helically complexed lipids could inhibit starch decomposition. However, the high thermal stability of cassava and triticale starch suggested that the property of starch matrix could also be involved as the stabilizing factor.

The analysis of the UV-VIS absorption spectra of iodine complexes of resulting dextrins involved the 625 nm-band shift and the variation of the A_{625}/A_{545}

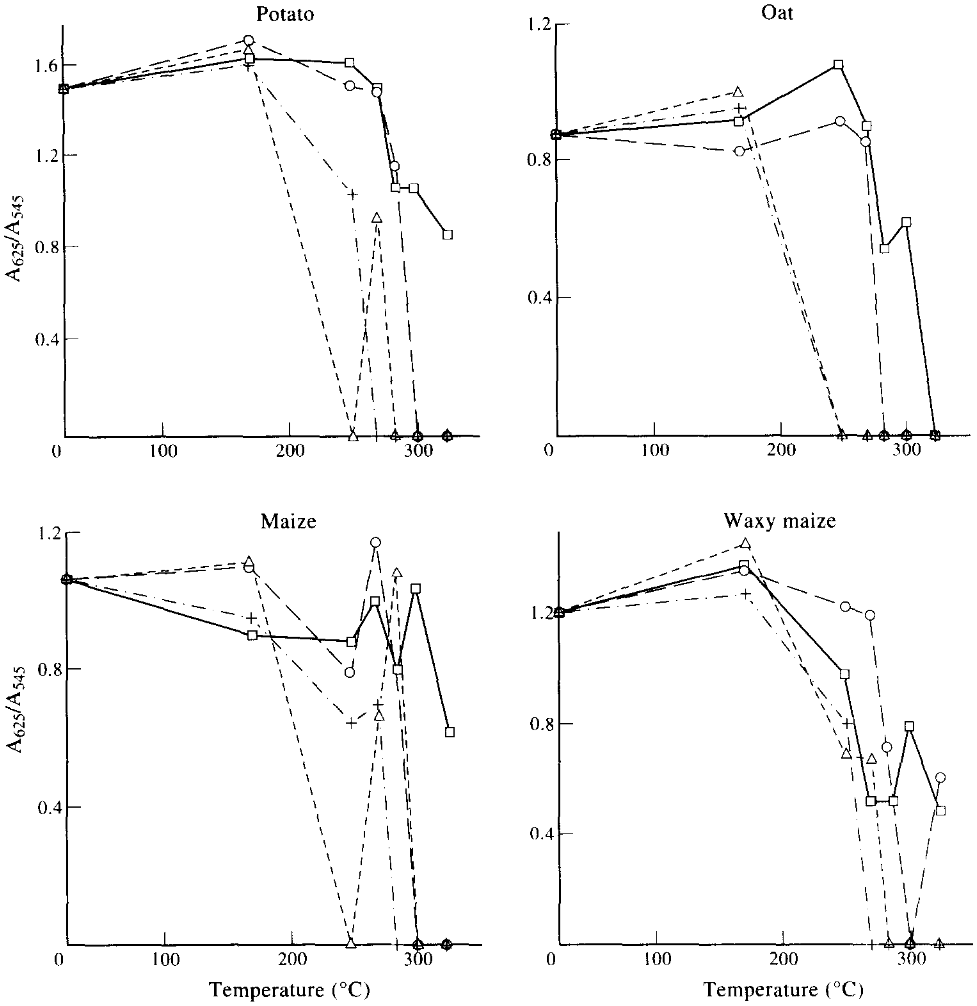


Fig. 3. The extent of thermal damage of potato, oat, maize and waxy maize starch presented as the function of heating temperature for a given period. The damage was recognized from the reaction of resulting dextrins with iodine. (30 min—squares and solid lines; 60 min—points and dotted lines; 90 min—triangles and pointed lines; 120 min—crosses and pointed-dotted lines).

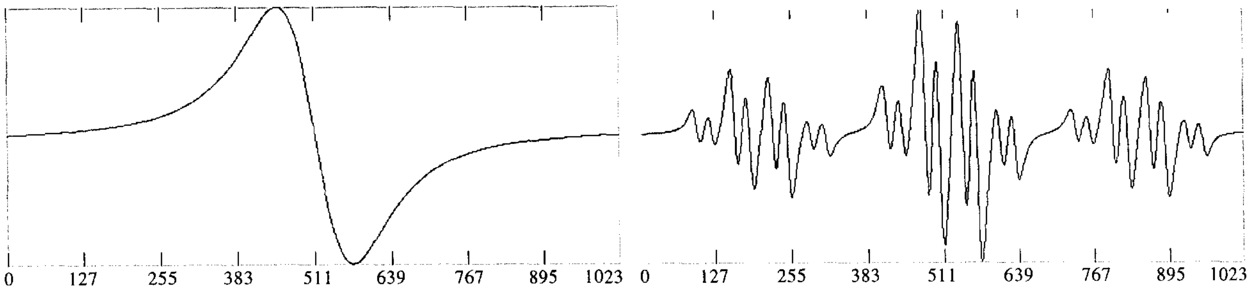


Fig. 4. The EPR spectra simulated under assumption of intermolecular interactions (left) and lack of such interactions (right). The left spectrum is common for all structures assumed in Fig. 1 whereas the right spectrum relates to structure no. 10.

ratio. The band-shift from the original 626, 583, 598 and 614 nm for potato, oat maize and waxy maize starch, respectively, to even 515 nm (Tomasik *et al.*, 1996), reflects the extention of the damage of amylose which produced shorter chain dextrins. The decreasing A_{625}/A_{545} ratio does not mean that amylose decomposed prior to amylopectin because the blue shift of this band is a function of increasing concentration of short chain dextrins, regardless of whether they originated from amylose or amylopectine. On the contrary, the increase of the A_{625}/A_{545} ratio suggests that amylopectin decomposed prior to amylose. This situation was observed for all four tested starch varieties, particularly at lower temperatures and at the start of the reaction. One may also see that the extention of the damage of starch matrix, as well as its macromolecules, was not proportional to time or to temperature. The variation of the A_{625}/A_{545} ratio varied from one starch variety to another (see Fig. 3). This could be due to a different distribution of both components in the starch granules of various starch varieties.

The radicals from starch were unusually stable. They survived a several months-long air exposure and extraction with hydroxylic solvents such as cold and hot water as well as hot ethanol, although in an extreme case the concentration of unpaired spins decreased even by up to 80%. The comparison of the effect of cold water treatment followed by evaporation to dryness without filtration with the effect of ethanolic extraction (Fig. 2, spectra 2 and 5, respectively), suggests that unpaired spins were localized mainly in the extractable, low molecular fraction of dextrins. The stability of radicals can be explained as the result of either perfect delocalization of unpaired spins within the glucose unit radicals and/or steric hindrance of the unpaired spins from the approaching spin scavenger. The resemblance of experimental spectra to those simulated under the assumption of intermolecular spin–spin interactions and the lack of such similarity, when this assumption was rejected, pointed to an essential role of a migration of unpaired spins within the structure in the stabilization of radicals.

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