



Influence of copper catalyst on the mechanism of carbohydrate radicals generation in oxidized potato starch

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ABSTRACT

Using EPR technique it was stated that copper ions introduced into potato starch, as catalyst of its oxidation with H_2O_2 , was the active reagent in the generation of stable carbohydrate radicals. EPR parameters indicated that radicals, thermally generated in starch oxidized with H_2O_2 in the presence of $Cu(II)$, were formed by abstraction of hydrogen atom from C(5) atom of glucose unit. It was found that generation of radicals was correlated with the reduction of $Cu(II)$ to $Cu(I)$ and formation of glucose-copper complexes. The reduction of copper occurring during thermal treatment was confirmed by colour parameters (a' increased from 0.96 for native starch to 9.10 for starch oxidized with H_2O_2 in the presence of copper catalyst). In the starch not containing copper (native and oxidized with H_2O_2) different types of radicals were generated. The mechanism of their creation consisted in abstraction of hydrogen from C(6), C(1) and C(3) carbon atoms. Formation of radicals upon dehydration of starch was also considered. Generation of radicals was correlated with the amount of copper catalyst used to oxidation process (0.1–0.3%) and with the number of carboxyl groups which was equal to 0.12% in starch oxidized with H_2O_2 and increased to 0.22% in starch oxidized in the presence of copper catalyst (0.3% of Cu). The stability of carbohydrate radicals was influenced mainly by distortion of the starch structure, as well as the presence of copper ions. Increase of the content of carbonyl groups upon oxidation (to 0.13% for starch oxidized with H_2O_2 and 1.31% for starch oxidized with H_2O_2 in the presence 0.3% copper catalyst) pointed to the progressive degradation of starch structure during oxidation.

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1. Introduction

Oxidation of starch is one of the methods of its modification, which improves its properties adjusting them to technological requirements and needs of consumers. Oxidation of starch results in the formation of $-CHO$ and $-COOH$ groups and the partial depolymerization (Forsell, Hamunen, Autio, Suorti, & Poutanen, 1995; Pietrzyk & Fortuna, 2005; Zhu & Bertoft, 1997). It was stated that physicochemical properties which modify oxidized starch structure, such as number of carbonyl and carboxyl groups and the level of depolymerization, depend mainly on the botanical origin of native starch, the kind of oxidizing agent, the reaction conditions and the size of starch granule (Forsell et al., 1995; Kuakpetoon & Wang, 2001; Manelius, Buleon, Nurmi, & Bertoft, 2000; Sangseethong, Lertphanich, & Sriroth, 2009; Sangseethong, Termvejsayanon, & Sriroth, 2010; Zhu & Bertoft, 1997; Zhu,

Sjoholm, Nurmi, & Bertoft, 1998; Zhang, Zhang, Wang, & Wang, 2009). The course of oxidation may be influenced by the content of amylose and amylopectine (Parovuori, Hamunen, Forsell, Autio, & Poutanen, 1995; Kuakpetoon & Wang, 2006; Wing & Willett, 1997). From among various oxidation reagents hydrogen peroxide is especially preferred because it does not give waste products adversely affecting the environment (Isbell & Frush, 1987; Tolvanen et al., 2009, 2011). Efficiency of oxidation process can be improved by introduction of catalyst, e.g. metal ions such as $Cu(II)$, $Fe(II)$, $V(V)$ or $W(VI)$; however, possibility of their interaction with starch matrix should be taken into account (Achremowicz, Gumul, Bala-Piasek, Tomasik, & Haberkowicz, 2000; Bala-Piasek & Tomasik, 1999; Ciesielski & Tomasik, 2004; Ciesielski, Lii, Yen, & Tomasik, 2003; Harmon, Gupta, & Jahnsen, 1971; Manelius et al., 2000; Parovuori et al., 1995; Pietrzyk, Fortuna, & Sowa, 2006; Śmigielńska & Lewandowicz, 2007; Śmigielńska, Lewandowicz, Goslar, & Hoffmann, 2005; Tolvanen et al., 2009, 2011).

Thermal treatment performed under air can additionally influence physicochemical properties of starch. It frequently leads to the formation of radical species, which can be considered as one of the stages of starch degradation (Ciesielski & Tomasik, 1997; Tomasik

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& Jane, 1995; Tomasik, Pałasiński, & Wiejak, 1989). The process of radical generation may be affected by the type of starch structure. It can be also controlled by the temperature of heating, the manner of starch modification, the presence and the concentration of functional groups such as $-\text{COOH}$, $-\text{CHO}$, $-\text{PO}_4^{3-}$, the presence of additional substances, e.g. sweeteners or metal ions introduced as supplements or as catalysts of chemical processes (Bidzińska, Dyrek, Fortuna, Łabanowska, & Pietrzyk, 2004; Blennow et al., 2006; Łabanowska et al., 2008, 2009; Pietrzyk et al., 2006; Śmigielńska & Lewandowicz, 2007; Śmigielńska et al., 2005; Tolvanen et al., 2009, 2011).

The paper presents the influence of copper ions, introduced as a catalyst of oxidation of the potato starch with hydrogen peroxide, on the mechanism of the thermal radical formation. Although hydrogen peroxide contains high amount of active oxygen and shows high oxidative potential, it is not very reactive towards organic functional groups. Therefore, the addition of transition metal ion catalysts is needed to enhance the process (Isbell & Frush, 1987). In the presence of iron or copper catalyst the reaction of starch oxidation occurs by radical mechanism with the formation of very reactive OH^\bullet species. On the other hand, thermal treatment of starch is accompanied by the generation of stable radical species. The earlier studies (Łabanowska et al., 2008) showed that the introduction of copper salts to reaction medium containing starch led to the interaction between Cu^{2+} and hydroxyl or carboxyl groups of the starch resulting in the formation of different kinds of copper complexes. The electron paramagnetic resonance (EPR) technique was very useful to detect both the copper ions and the radical species because of their paramagnetic character. The study of radicals behaviour is of particular interest in view of the damaging role played by reactive free radicals in living cells (Ashok & Ali, 1999; Babbs, 1990; Finkel & Holbrook, 2000; Steinberg, 1995). On the other hand, the radicals with high stability can be considered as spin traps for dangerous short living paramagnetic species. The protective role of such long lived radicals was already confirmed in plant systems (Pearce et al., 1997). Hence, studies of the nature of radicals generated thermally in the starch, material which is often subjected to treatment at higher temperature, is of substantial practical significance.

In the present work the EPR technique has been applied to investigate the process of starch oxidation with H_2O_2 upon addition of copper catalyst. The study concentrated on the type of interaction of copper ions with starch matrix, on the starch structure evolution upon thermal treatment and on the nature of radicals formed during this process.

2. Materials and methods

2.1. Materials

The potato starch “Superior” produced by PEPEES S.A. (Łomża, Poland) was used for the study.

Oxidation of starch was performed in an acid environment, by hydrogen peroxide alone or with the addition of aqueous solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (pro analysis, Merck, Darmstadt, Germany). Water solution of copper salt corresponding to: 0.1, 0.2 and 0.3 g of $\text{Cu}^{2+}/100 \text{ g dm}^{-3}$ starch was added to 42% starch suspension in water, according to the method described by Parovuori et al. (1995). The mixture was stirred at 250 rpm and then heated for 15 min at a temperature 313 K. After that, 30% solution of H_2O_2 (analytically pure; POCh, Gliwice, Poland) was added dropwise to the reaction mixture to achieve the final H_2O_2 concentration of $2 \text{ g}/100 \text{ g dm}^{-3}$ starch. After 60 min the mixture was washed with distilled water (500 cm^3), dried at a temperature of 293 K for 48 h, pulverised, and passed through 0.2 mesh sieve.

2.2. Content of carboxyl group, carbonyl group and copper

Carboxyl group content was determined according to the standard ISO 11214 (1996). Carbonyl group content was estimated according to Whistler, BeMiller, & Paschall (1967). The content of copper in the starch samples was determined by flame atomic absorption spectroscopy method using an Avanta Sigma spectrometer (GBC, Australia). The wet mineralization of the samples with HNO_3 and H_2SO_4 was performed at 523 K in a Wet Digester B-400 (Büchi) mineralizer.

2.3. Colour parameters

Colour parameters in the CIE $L^*a^*b^*$ system (measuring geometry $d/8^\circ$, illuminant D65, observer 10°) were measured using a Color i5 spectrophotometer (X-Rite, USA).

2.4. Thermal treatment

Temperature of thermal treatment was chosen taking into account the technological conditions used in baking processes. The samples of starch (about 20 mg) were placed in the quartz EPR tubes and treated in air for 30 min at 433 K, followed by 30 min at 483 K. Some samples were subjected also to 30 min thermal treatment at 503 K. Before recording of the EPR spectra the tubes with the samples cooled to room temperature and closed with a paraffin membrane.

2.5. EPR measurements

EPR measurements were carried out on an X-band Bruker ELEXSYS 500 spectrometer (Karlsruhe, Germany) with 100 kHz field modulation. The spectra were recorded at 293 K and 77 K with modulation amplitude of 0.5 mT and 0.1 mT before and after thermal treatment of the samples. They were registered at microwave powers: 0.3, 3.0 and 10.0 mW. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a g-factor standard. EPR parameters such as g factor value, hyperfine splitting constant A, peak-to-peak line width ΔB_{pp} were found by a simulation procedure, using a modified program SIM 14 (Lozos, Hoffman & Franz). The accuracy of determination of EPR parameters was ± 0.005 and ± 0.0005 for g values of Cu^{2+} and radical signals, respectively. The accuracy of estimation of hyperfine splitting constant was ± 0.5 and $\pm 0.1 \text{ mT}$ for parameters A of copper ions and radical species, respectively. The integral intensity of copper signals was related to the same mass of the samples and was compared with the integral intensity of the signal of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}/\text{K}_2\text{SO}_4$ with the known number of spins (2.90×10^{16} spins/g). The estimation of the amount of radicals was made using the DPPH as a reference sample with the number of spin equal to $0.97 \times 10^{15}/\text{g}$.

3. Results and discussion

No EPR signals are observed in the native potato starch before its modification. The introduction of copper ions into the starch, as a catalyst of oxidation, leads to the appearance of the complex copper spectrum with integral intensity strongly depended on the contents of copper ions in the sample (Figs. 1 and 2). The amount of Cu^{2+} ions visible in EPR spectra is equal to $2.5\text{--}3.6 \times 10^{17}/\text{g}$ of starch, which is about 6–8 times lower than that found analytically in the oxidized starch samples containing 0.1–0.3% of copper, respectively (Fig. 2). Such discrepancy between amounts of copper ions estimated by two methods strongly suggests that part of copper species not detected by EPR is clustered and antiferromagnetically coupled (Solomon, 1983). Indeed, adsorption of copper ions on the surface of starch granules was observed by Achremowicz et al. (2000). The

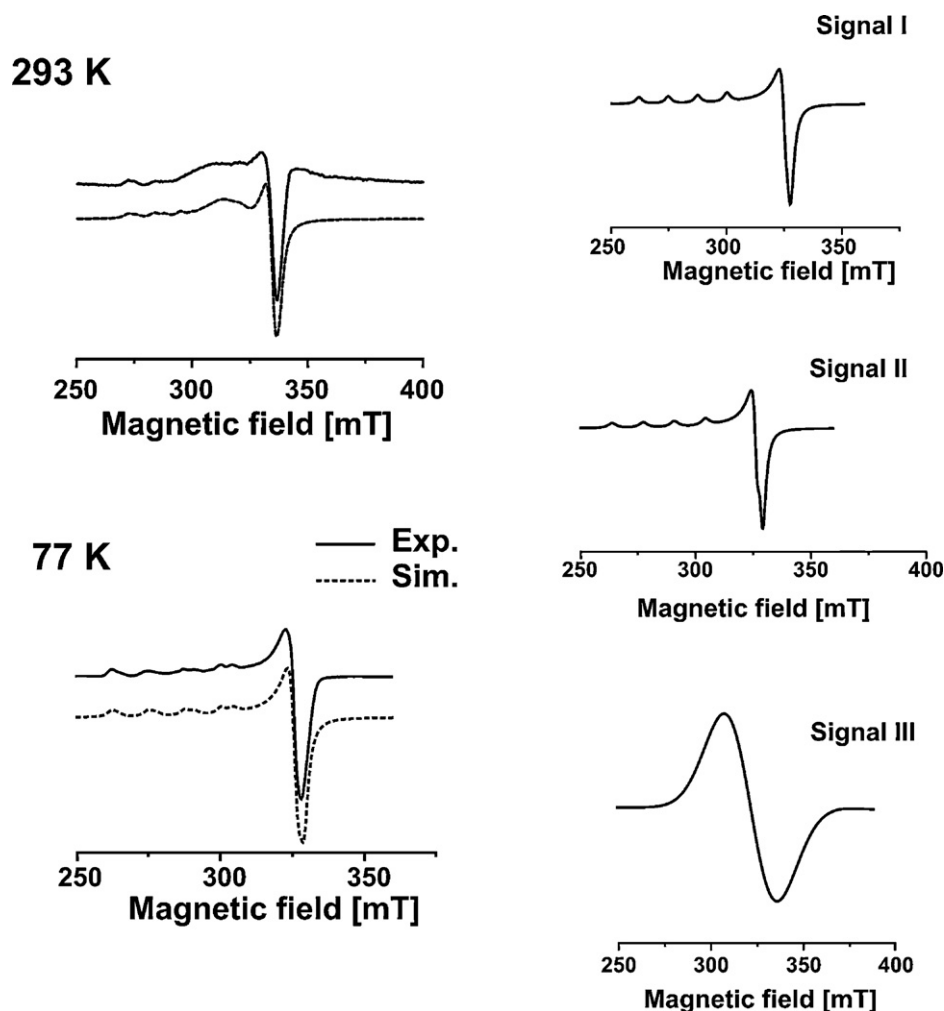


Fig. 1. Experimental and simulated EPR spectra (registered at 293 K and 77 K) of Cu^{2+} ions in the potato starch oxidized with H_2O_2 before thermal treatment at 483 K, and particular signal components.

simulation of copper spectrum registered at 293 K reveals the presence of three paramagnetic centres (I, II and III) with parameters pointing to the existence of copper species with different surroundings, formed by interaction of copper ions with functional groups of the starch (Table 1). The signals of centres I and II exhibit axial symmetry and resolved hyperfine structure (HFS) around g_{\parallel} imply-

ing the presence of two kinds of isolated Cu^{2+} species with slightly different surroundings. These centres are limited in their mobility by interactions with oxygen atoms of the starch matrix. Their EPR parameters (Table 1) clearly show that copper ions are surrounded by elongated octahedron of oxygen ligands (Abragam & Bleaney, 1986). Similarity of EPR parameters of both I and II centres to those characteristic for hexaaqua complexes of copper (Lewis, Alei, & Morgan, 1966) point to the presence of water molecules in coordination sphere of copper–starch complexes.

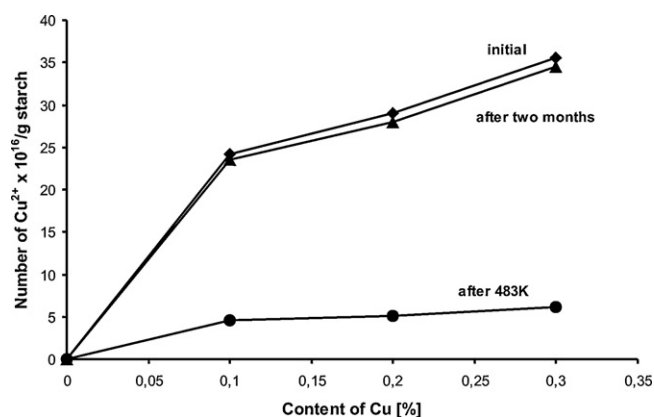


Fig. 2. The dependence between the number of Cu^{2+} ions visible in EPR spectra and the amount of copper ions used as catalyst (♦ – before thermal treatment, ● – after thermal treatment, and ▲ – after two month storage of the sample at room temperature).

Table 1
EPR parameters of Cu^{2+} ions introduced into the potato starch as catalyst of oxidation. Spectra registered at 293 and 77 K before thermal treatment.

Temp. (K)	Parameter	I	II	III
293	g_{\parallel}	2.410	2.370 (2.364 ^a)	
	g_{\perp}	2.087	2.080 (2.083 ^a)	
	g_{av}	2.195	2.177 (2.177 ^a)	2.157
	A_{\parallel} (mT)	11.4	12.4 (12.4 ^a)	
77	g_{\parallel}	2.411	2.380 (2.377 ^a)	
	g_{\perp}	2.085	2.078 (2.079 ^a)	
	g_{av}	2.194	2.179 (2.177 ^a)	
	A_{\parallel} (mT)	12.7	13.4 (12.7 ^a)	

Maximum error of g : ± 0.005 .

Maximum error of A : ± 0.5 mT.

^a EPR parameters of Cu^{2+} introduced into corn starch without phosphate groups (Blennow et al., 2006).

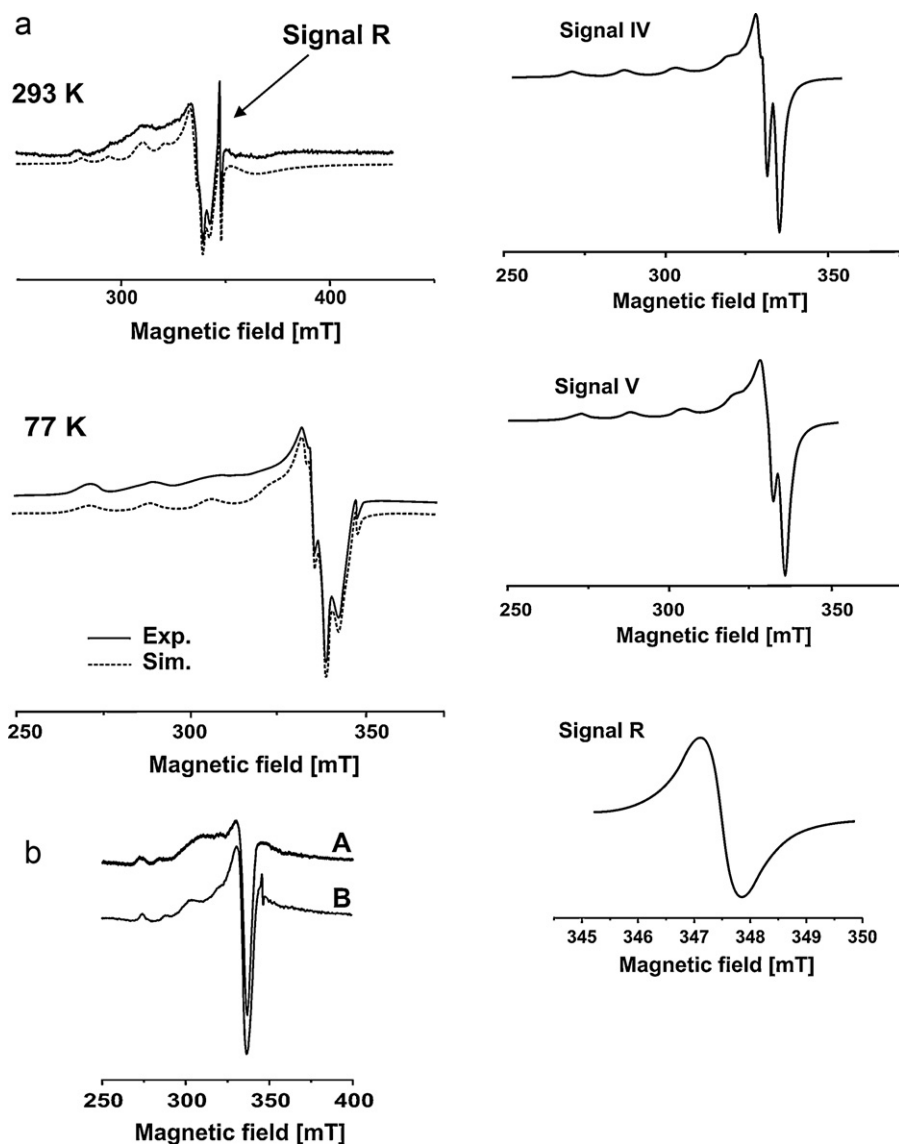


Fig. 3. (a) Experimental and simulated EPR spectra (registered at 293 K and 77 K) of Cu^{2+} ions in the potato starch oxidized with H_2O_2 after thermal treatment at 483 K, and particular signal components. (b) Experimental EPR spectra of Cu^{2+} registered at 293 K. A, before thermal treatment. B, after two-month storage at room temperature of thermally treated oxidized starch.

The comparison of parameters of the centre I with those reported in paper (Blennow et al., 2006) strongly suggests that the copper ion is bonded to the starch matrix by an oxygen atom of the phosphate group. The parameters of signal II, whose contribution to the integral intensity of the overall spectrum is larger than that of signal I, are practically the same as those found for corn starch not containing phosphate groups, in which the interaction of copper ion with starch matrix occurs by oxygen atom of the hydroxyl or carboxyl group of glucose unit (Table 1). Therefore, it is assumed, that copper responsible for signal II is bonded in a similar manner. The isotropic signal III with $g = 2.1572$ and $\Delta B_{pp} = 11$ mT is typical for freely rotating $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ complex in starch (Łabanowska, Bidzińska, Dyrek, & Szymońska, 2006). At 77 K the rotation of the copper species stops and the isotropic signal III becomes anisotropic and overlaps with signals I and II (Fig. 1).

After thermal treatment of the oxidized potato starch, the significant changes occur in the EPR spectrum (Fig. 3a). The integral intensity of copper signals strongly decreases. The heating results in the removal of water and disappearance of the $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ signal (signal III). In the spectrum registered at room temperature a

new intensive narrow line at $g = 2.005$ (signal R) becomes visible. Its intensity decreases with lowering of the temperature, contrary to the behaviour of copper signals, which grow as the temperature falls. The simulation procedure allows for discrimination of two copper signals, IV and V, with EPR parameters different from those observed in the spectrum before heating of starch (Table 2), indicating the formation of new kinds of copper–starch complexes. Signals IV and V exhibit axial symmetry, but the lowering of g tensor values together with increase of $A_{||}$ values, in comparison with those observed for signals I and II, points to the shortening of the equatorial copper–ligands bonds (Łabanowska et al., 2008). In addition, a decrease of the g perpendicular component value is observed in signal V. Moreover, well separated hyperfine lines around g_{\perp} become visible in both signals (Fig. 3a, Table 2). The similarity of EPR parameters of signals IV and V to those found for copper species introduced into the native starch (Łabanowska et al., 2008) allow for assignment of these signals to hexacoordinated copper species bonded to starch matrix by two oxygen atoms and copper ions surrounded by oxygen ligands in square planar coordination, respectively. The characteristic feature of copper signals is a strong

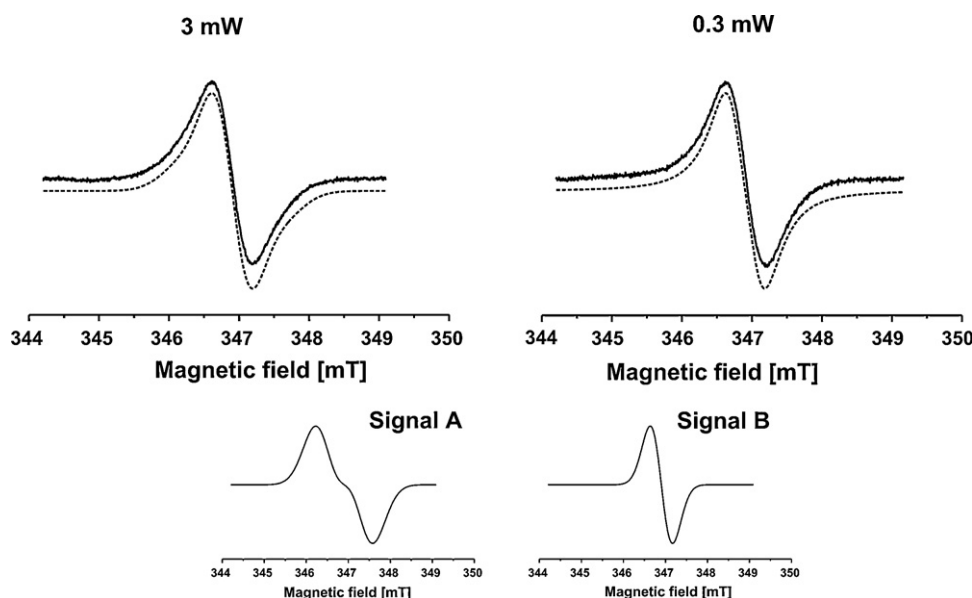


Fig. 4. Experimental and simulated EPR spectra (registered at 293 K at 3 and 0.3 mW) of radical species in the potato starch oxidized with H_2O_2 in the presence of copper catalyst after thermal treatment at 483 K, and particular signal components.

Table 2

EPR parameters of Cu^{2+} ions introduced into the potato starch as catalyst of oxidation. Spectra registered at 293 and 77 K after thermal treatment at 483 K.

Temp. (K)	Parameter	IV	V
293	g_{\parallel}	2.317	2.307
	g_{\perp}	2.077	2.059
	g_{av}	2.157	2.142
	A_{\parallel} (mT)	15.4	15.4
	A_{\perp} (mT)	0.9	0.9
77	g_{\parallel}	2.321	2.308
	g_{\perp}	2.080	2.062
	g_{av}	2.160	2.144
	A_{\parallel} (mT)	15.5	15.6
	A_{\perp} (mT)	0.9	1.0

Maximum error of g : ± 0.005 .

Maximum error of A : ± 0.5 mT.

decrease, about six times, of their integral intensity after thermal treatment of the starch (Fig. 2). This phenomenon is accompanied by the simultaneous appearance of the narrow intensive signal R with $g_{\text{av}} = 2.005$ (Fig. 3). The recording of this signal in the narrow magnetic field range (5 mT) reveals its complex structure, characteristic of starch radicals (Fig. 4, Table 3). The intensity of this signal grows with the increase of the copper catalyst content (Fig. 5a). It is paralleled by the increase of the content of carboxylic groups (Fig. 5b), whose formation is also a function of the amount of copper

ions introduced as catalyst of starch oxidation (Fig. 5b, insert).

The oxidation of starch in the presence of copper catalyst strongly influences colour parameters of the samples subjected to thermal treatment (Table 4). The most characteristic feature is a significant increase of a^* parameter for samples oxidized in the presence of copper, which indicates an essential contribution of the red component in the visible spectrum. Such result can suggest the increase of the concentration of Cu^+ species, which exhibit the red colour in the oxygen surroundings, and is in agreement with the observed lowering of the Cu^{2+} ions EPR signal intensity. The reduction of Cu^{2+} to diamagnetic Cu^+ ions during thermal treatment of starch was earlier confirmed by ESCA measurements (Łabanowska et al., 2008).

Both processes occurring during thermal treatment of starch samples: the reduction of Cu^{2+} to Cu^+ and simultaneous formation of radical species were discussed in detail by Łabanowska et al. (2008). Their interdependence: increase of the radical number with increasing content of reduced copper ions is presented in Fig. 6.

The radicals generated upon thermal treatment of starch exhibit unusual stability and their EPR characteristics point to their carbohydrate nature. The simulation procedure performed under assumption of the Gaussian character of the signals reveals two components of the spectra: a doublet with $A = 0.7\text{--}0.9$ mT and $g = 2.0035$ (signal A) and a more intensive isotropic single line with $g = 2.0035$ (signal B) (Fig. 4, Table 3). The splitting value of signal

Table 3

EPR parameters of radicals generated at 483 in potato starch native and oxidized with H_2O_2 , in the presence and without of Cu^{2+} ions as catalyst. Registration of EPR spectra at 293 K.

		Signal A		Signal B	
		g	A (mT)	g	
Oxidized potato starch + Cu ²⁺		$g = 2.0035$	$A = 07 - 0.9$	$g = 2.0035$	
Signal X		Signal Y		Signal Z	
Native potato starch	$g = 2.0061$	$A_{\alpha} = 1.18A_{\beta} = 0.60$	$g = 2.0051$	$A_{\beta} = 0.45$	$g = 2.0062$
Oxidized potato starch	$g = 2.0061$	$A_{\alpha} = 1.18A_{\beta} = 0.60$	$g = 2.0051$	$A_{\beta} = 0.45$	$A_{\beta} = 2.80$

Maximum error of g : ± 0.0005 .

Maximum error of A : ± 0.1 mT.

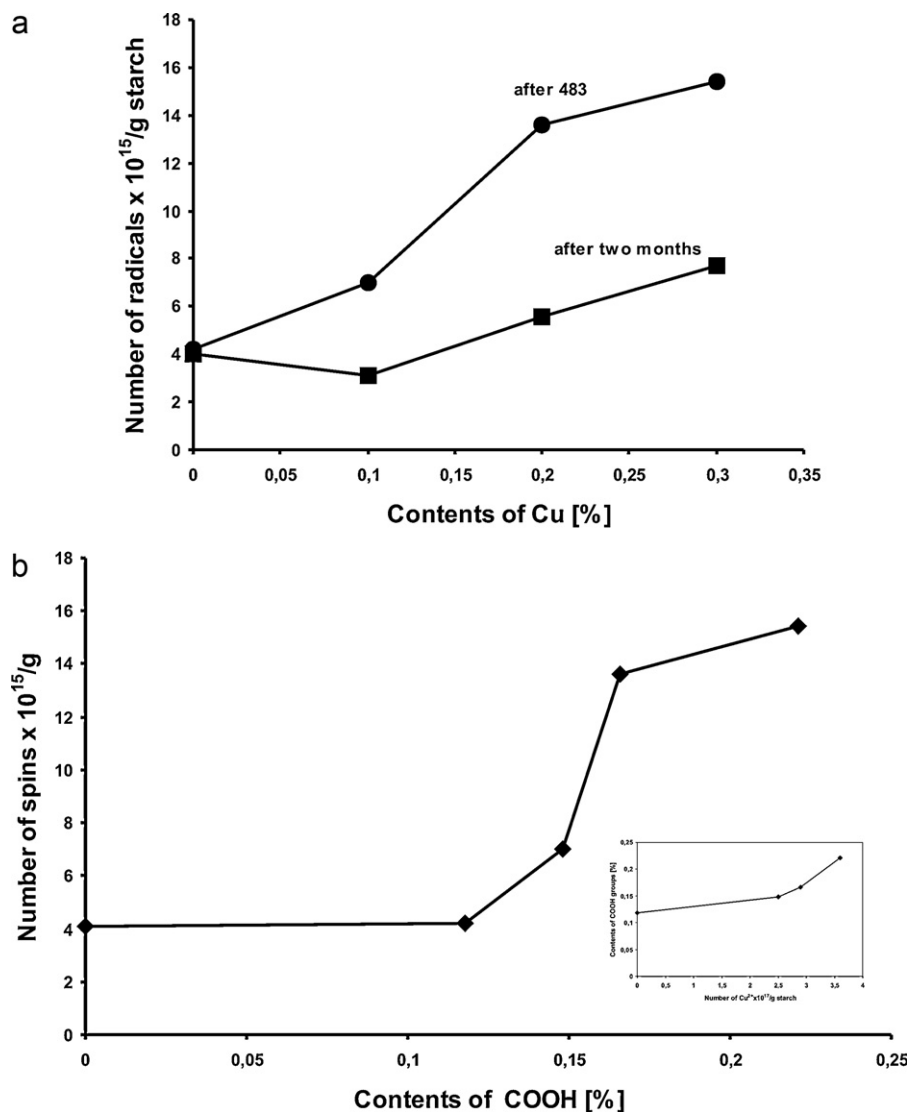


Fig. 5. (a) The dependence between the number of generated radicals and the amount of copper ions used as catalyst (● – after thermal treatment and ▲ – after two month storage of the sample at room temperature). (b) The dependence between the number of generated radicals and the amount of carboxylic groups in oxidized starch. (Insert) The dependence between the amount of carboxylic groups and the number of copper ions giving EPR signals in oxidized potato starch.

A depends on the content of copper in starch, decreasing with its increase. The parameters of signals A and B are similar to those found for radical species generated thermally in potato starch containing copper ions and attributed to carbohydrate radicals with an unpaired electron localized at carbon atom of glucose unit (Łabanowska et al., 2008). The value of hyperfine splitting, originating from interaction between magnetic moments of an unpaired electron and nuclear spin of hydrogen ($I = 1/2$) is smaller for the signal registered in the sample subjected to thermal treatment, than that found for radical generated mechanically in amylose (Kuzuya,

Yamauchi, & Kondo, 1999). The effect is most likely caused by the weakening of hyperfine interactions due to deformation of starch structure and elongation of bonds in glucose units occurring during oxidation process and thermal treatment. After two-month storage at room temperature, signals A and B decrease about two times (Fig. 5a). Simultaneously, the copper signals regain their intensity (Figs. 2 and 3b). It is worth-while noticing that only two signals: II and III become restored. The growth of signal III is due to the rehydration of starch, while the reappearance of signal II, related to the copper ion bonded to oxygen of starch matrix, proves the participa-

Table 4

Colour parameters of potato starch after thermal treatment at 483 K as dependent on oxidation and catalyst addition.

Starch	Colour parameters			
	<i>L</i> [*]	<i>a</i> [*]	<i>b</i> [*]	ΔE
Native	89.92 ± 0.14 ^a	0.96 ± 0.08 ^a	14.90 ± 0.17 ^a	References ^a
Oxidized (H ₂ O ₂)	90.25 ± 0.21 ^a	0.90 ± 0.10 ^a	14.23 ± 0.37 ^a	0.56 ± 0.25 ^a
Oxidized (H ₂ O ₂ 0.1% Cu(II))	77.72 ± 0.09	5.84 ± 0.05	25.56 ± 0.09	279.98 ± 11.50
Oxidized (H ₂ O ₂ 0.2% Cu(II))	67.52 ± 0.10 ^b	9.63 ± 0.61 ^b	28.30 ± 0.18	756.49 ± 25.21
Oxidized (H ₂ O ₂ 0.3% Cu(II))	67.50 ± 0.11 ^b	9.10 ± 0.08 ^b	22.56 ± 0.09	627.59 ± 15.21

Means followed by the same letters are not significantly different at $\alpha = 0.05$.

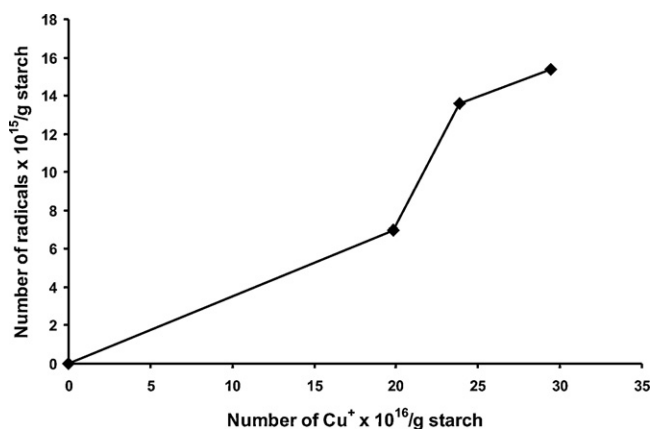
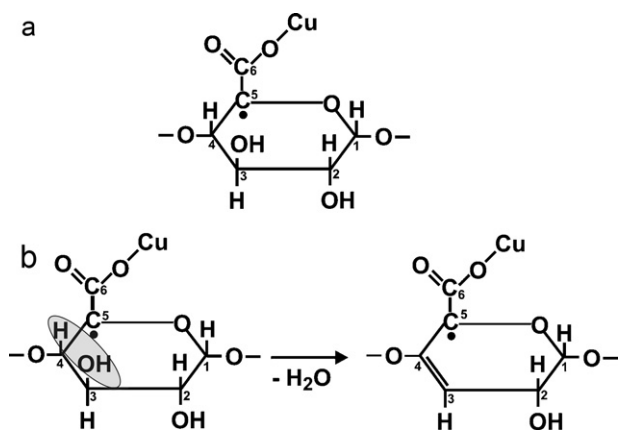


Fig. 6. The dependence between the number of generated radicals and the amount of reduced copper ions.

tion of these copper species in the radical generation process. Such interrelation between intensities of the signals of copper ions and carbohydrate radical species indicates that copper catalyst not only influences the oxidation process of the starch but also controls both the radical generation and its stability. It is known from literature that oxidation process engages mainly C(6), C(1) and C(3) atoms of glucose unit, leading to the creation of carboxyl group bonded to C(5) and/or to breaking of the bonds at C(1) and C(3) with formation of carbonyl group (Wurzburg, 1986). The dependence between the intensity of the radical spectrum and the content of carboxyl groups confirms the interrelation between both processes: oxidation and radical generation (Fig. 5b). In view of the fact that the formation of radicals, localized at carbon atom of glucose unit, is associated with the reduction of Cu^{2+} to Cu^+ , we consider the transformation, during thermal treatment, of glucose- $\text{COO}-\text{Cu}^{2+}$ complexes to glucose- $\text{COO}-\text{Cu}^+$ ones. In such system the radical species are formed by the abstraction of hydrogen from C(5) (Fig. 8, Scheme 1a). The proposed mechanism of this process was presented in (Łabanowska et al., 2008). The magnetic moment of an unpaired electron localized at C(5) interacting with nuclear spin of β hydrogen at C(4) should lead to a hyperfine doublet (signal A, Scheme 1a). As to the origin of signal B, it should be noted that high temperature treatment (483 K) is accompanied by dehydration of the sample. This process, followed by the removal of hydrogen from C(4) and OH group from C(3), leads to the formation of unsaturated bond between C(4) and C(3) atoms. The lack of H atom at C(4) means lack of the source of hyperfine interactions. As a result, a single EPR



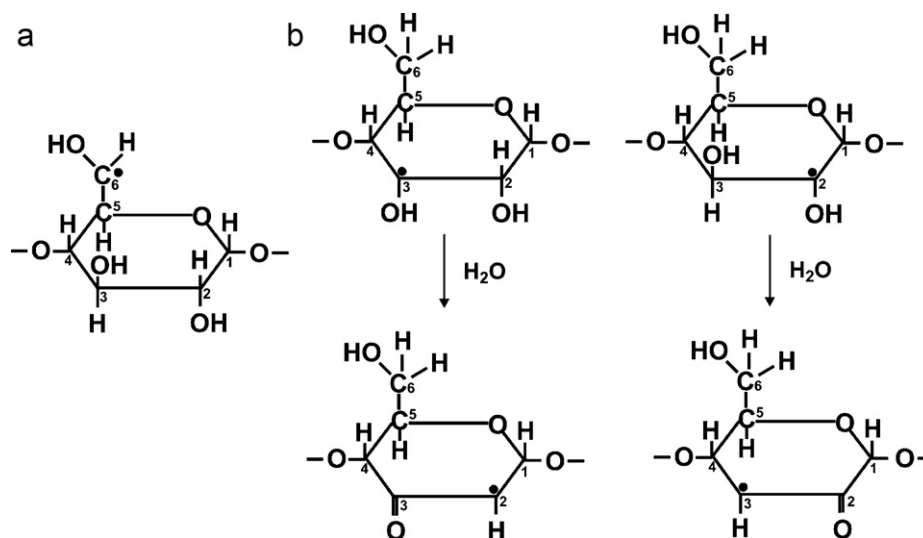
Scheme 1. (a) The structure of radical generated in oxidized starch in the presence of copper catalyst (radical C(5)). (b) The structure of radical generated in oxidized starch in the presence of copper catalyst after dehydration (radical C(5)).

line (signal B, Fig. 8, Scheme 1b) is observed. With increasing temperature of thermal treatment (503 K) the dehydration process is more effective and the signal with hyperfine structure disappears completely.

After thermal treatment, both the native and the oxidized starch without Cu^{2+} , show very similar EPR spectra, although, different from those recorded for radicals formed in the starch containing copper. The spectra exhibit more complex hyperfine structure and g factors shifted slightly towards higher values (Fig. 7, Table 3). The registration of the spectra at two different microwave powers reveals the same saturation ability of all lines, confirming similar character of responsible radical species. The number of spins related to carbohydrate radicals is similar for both kinds of starch, native and oxidized. This shows that oxidation performed without copper catalyst does not influence the process of radical generation in starch as strongly as the presence of copper does. The simulation procedure confirms this observation. Assuming Gaussian character of the lines, the simulation reveals the presence of three signals: X, Y and Z. The signal X consists of four lines around $g=2.0061$ with similar intensities, indicating the interaction of the magnetic moment of an unpaired electron with magnetic moments of two non equivalent hydrogen nuclei. Such situation may be due to the abstraction of hydrogen from C(6) atom of glucose unit and interaction of an unpaired electron, localized at this carbon atom, with α hydrogen at C(6) and β hydrogen at C(5) of starch (Fig. 8, Scheme 2a). Similar radical model was proposed on the basis of EPR-ENDOR studies, performed for thermally treated and X-ray irradiated α -D-glucose single crystals by Madden and Bernhard (1979, 1982) and confirmed by density functional theory calculations (Pauwels, Van Speybroeck, & Waroquier, 2006). In this model the weak interaction of an unpaired electron with hydrogen of hydroxyl group at C(6) was also taken into account. Such weak interaction, however, is not observed in the powder spectrum of starch sample investigated in the present study. The values of splitting equal to 1.18 mT and 0.60 mT found for signal X are lower than those reported for α and β hydrogen, respectively, in single crystal of glucose (Madden & Bernhard, 1979, 1982), probably because of the stronger deformation of starch structure occurring during thermal treatment at higher temperature. Starch dehydration at the relatively high temperature of radical generation should be also considered as a possible cause of the four line signal appearance. Yamauchi, Sugito, and Kuzuya (1999), who studied plasma induced radicals in polycrystalline glucose, proposed that dehydration induced transformation of primary hydroxyalkyl radicals, formed by abstraction of hydrogen from C(2) or C(3) carbon atoms, to acylalkyl radicals, and, in consequence, led to a four line signal (Fig. 8, Scheme 2b). In these centres magnetic moments of unpaired electrons interact with nuclear spins of α and β hydrogen atoms (Scheme 2b). However, in contrast to glucose molecules, in ordered starch structure the hydrogen atoms at C(3) and C(2) are directed towards the inside of the spiral structure of amylose or amylopectine, which hinders their abstraction from C(3) or C(2) (Kuzuya, Morisaki, Niwa, Yamauchi, & Xu, 1994). Indeed, no hydrogen abstraction was observed for radicals created mechanically in amylose (Kuzuya et al., 1999). Therefore, we reject the latter model and assign the four-lines signal X to the radical at C(6).

Signal Y with $g=2.0051$ and $A=0.45$ mT exhibits the highest intensity in the spectrum and is tentatively attributed to the radical at C(1) formed by abstraction of hydrogen from C(1) atom of glucose unit. In this centre a magnetic moment of an unpaired electron interacts with magnetic moment of β hydrogen at C(2) (Fig. 8, Scheme 3). The formation of radicals at C(1) atom in mono and polycarbohydrates was postulated by several authors (Abagyan & Apresyan, 2002; Yamauchi et al., 1999; Kuzuya et al., 1999).

In the spectra of both native and oxidized starch, registered at 3 mW, weak doublet around $g=2.0062$, split by 2.80 mT (signal Z),



Scheme 2. (a) The structure of radical generated in native and oxidized (without copper catalyst) starch (radical C(6)). (b) The proposed structure of radicals generated in native and oxidized (without copper catalyst) starch after dehydration (radicals C(3) and C(2)).

is also visible. We attribute this doublet to the radical species localized at C(3) (Fig. 8, Scheme 4). In this centre the interaction between magnetic moments of an unpaired electron and nuclei of β hydrogen atoms at C(4) and C(2) should occur. The HF constants values of interactions with β hydrogen at C(4) and C(2) for radical localized at C(3) in single crystal of glucose, very similar and equal to about 3.2 mT, have been reported in (Madden & Bernhard, 1979, 1982). In a powder spectrum such HF lines would overlap. In fact, only doublet with A constant equal to 3.04 mT was observed for C(3) plasma induced radical in polycrystalline glucose (Yamauchi et al., 1999). Small intensity of signal Z indicates low efficiency of the C(3) radical formation, which is in accordance with the above mentioned difficulties in abstraction of hydrogen from C(3) atom of glucose unit in starch structure.

During thermal treatment at higher temperature (503 K) signals X and Z disappear from the spectrum and only doublet Y with hyperfine splitting lower than that found at 483 K is observed.

The proposed models of the structure of radicals thermally generated in starch is an alternative to our previous interpretation (Dyrek et al., 2007), in which simulation procedure assumed creation of only one type of radical at C(1) with strong anisotropy of the signal. However, analysis of the experimental evidence shows that such a strong asymmetry is not encountered in carbohydrate radicals originating from glucose compounds (Kuzuya et al., 1999; Sasai, Yamauchi, Kondo, & Kuzuya, 2004; Yamauchi et al., 1999). Therefore, we believe that our current approach is more appropriate.

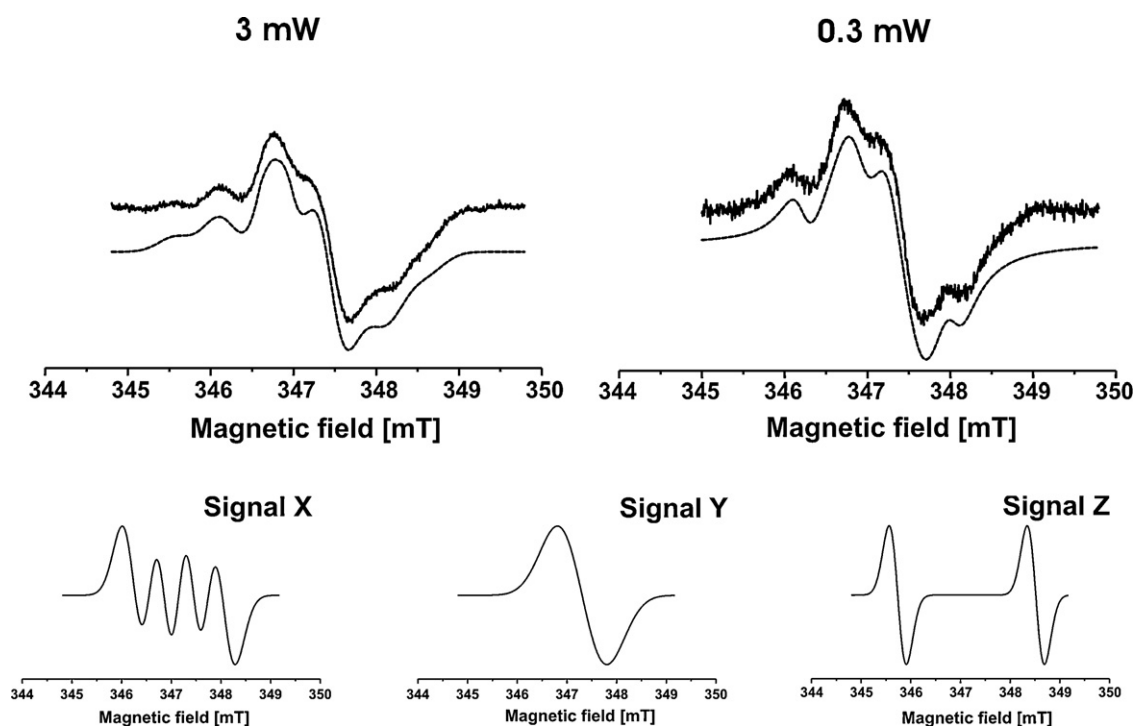
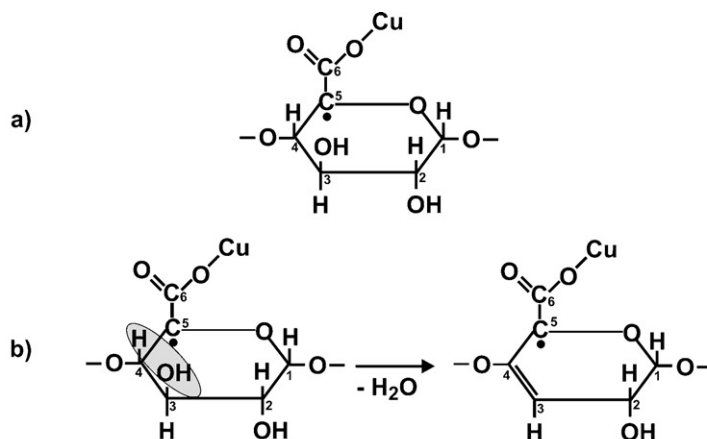
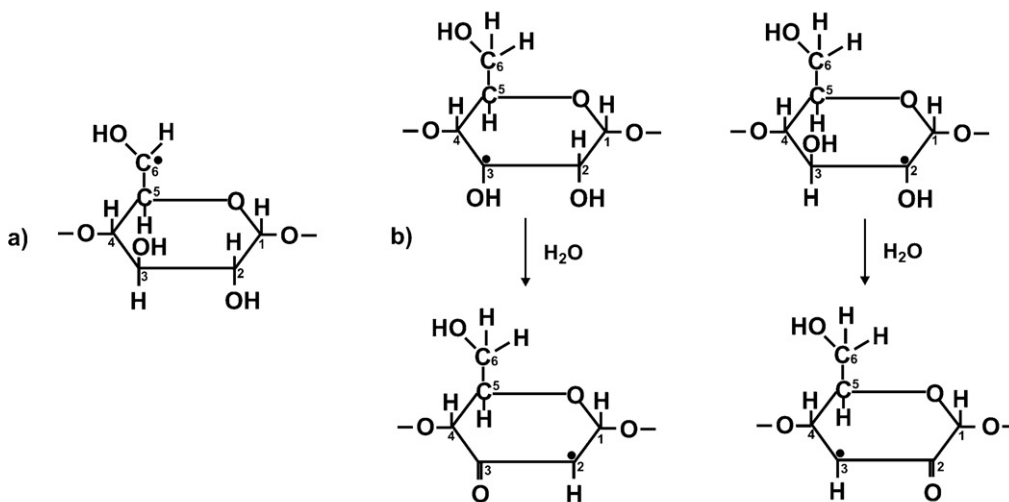


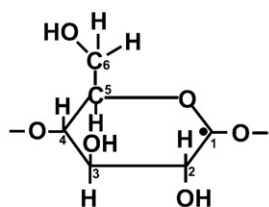
Fig. 7. Experimental and simulated EPR spectra (registered at 293 K at 3 and 0.3 mW) of radical species in the native potato starch after thermal treatment at 483 K, and particular signal components.



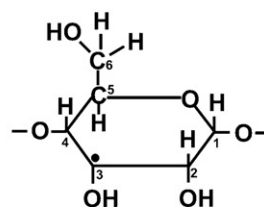
Scheme 1



Scheme 2



Scheme 3



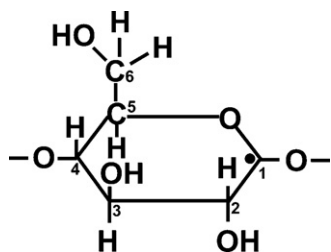
Scheme 4

Fig. 8. Schemes of structures of particular radical species generated thermally in potato starch.

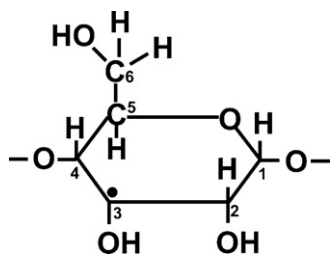
The characteristic feature of signals X, Y and Z, created upon heating of native and oxidized starch, is that the values of g factor are higher than those observed for radicals A and B, generated in the starch containing copper. To understand this phenomenon, it should be noted that in C(6), C(1) and C(3) radicals (X, Y and Z, respectively), generated in native and oxidized starch, the unpaired electron is localized at carbon atoms whose bonding to the neighbouring oxygen is shorter than the average C–O bond length in glucose (McDonald & Beevers, 1952; Molteni & Parrinello, 1997). On the other hand, in the case of the radical at C(5), created in the starch containing copper (signals A and B), the bond between angular oxygen atom and C(5) is longer than the average C–O distance

(Molteni & Parrinello, 1997). It is known that proximity of strongly electronegative oxygen leads to delocalization of the unpaired electron density from carbon to oxygen atom and causes increase of g parameter. Such influence of oxygen on carbon radicals was confirmed by DFT (density functional theory) calculations for radiation induced radicals in α -D-glucose (Pauwels et al., 2006). In view of the above, higher values of g factors observed for signals X, Y and Z, as compared with signals A and B, are due to larger electron delocalization over nearby oxygen atom.

Two months storage of the samples at room temperature resulted in a 80% decrease of signals associated with thermally generated carbohydrate radicals in native starch, whereas



Scheme 3. The structure of radical generated in native and oxidized (without copper catalyst) starch (radical C(1)).



Scheme 4. The structure of radical generated in native and oxidized (without copper catalyst) starch (radical C(3)).

the radicals present in oxidized starch, not containing copper ions, remained unaffected. In the oxidized starch containing copper, the radical signal shows intermediate stability and its decay to about 50% of original intensity is accompanied by a recovery of Cu(II) spectrum, mainly of signal II related to the copper–starch complex (Figs. 2 and 3b). The intrinsic viscosity of oxidized starch ($140\text{ cm}^3\text{ g}^{-1}$) is lower than that of native starch ($\sim 190\text{ cm}^3\text{ g}^{-1}$), which indicates an increase of depolymerization (Pietrzyk, 2011). It is likely that shortening of carbohydrate chains affects adversely electron pathways required for radical recombination and stabilizes radicals present in oxidized starch. In the case of copper-containing oxidized starch the depolymerization process is significantly more advanced which is reflected by ten-fold increase of carbonyl groups content in comparison with the starch oxidized without catalyst (0.12% of carbonyl groups), as well as a strong decrease of intrinsic viscosity ($\sim 60\text{ cm}^3\text{ g}^{-1}$) (Pietrzyk, 2011). In spite of the high depolymerization, the stabilization of radicals is less effective due to the existence of short range radical–copper interaction not affected by carbohydrate chain fragmentation. Therefore, two opposite processes: the stabilization of radicals by disordered starch structure and their decay connected with oxidation of Cu^+ control the level of radical species in the starch oxidized with H_2O_2 in the presence of copper catalyst.

4. Conclusions

Copper ions introduced to starch as catalyst of its oxidation are reactive agents during generation of radicals upon thermal treatment. The mechanism of the formation of carbohydrate radicals is connected with reduction of Cu^{2+} to Cu^+ . Copper ions interact with starch matrix forming various complexes, whose structures change upon conditions of treatment. These complexes can be monitored by EPR technique to yield information about changes occurring in starch structure. Radicals thermally generated in the starch containing copper are formed by abstraction of hydrogen atom from C(5) atom of glucose unit, whereas those found in starch not containing copper are created by abstraction of hydrogen from C(6), C(1) and C(3) carbon atoms. The distance between oxygen and carbon atom, at which an unpaired electron is localized, determines the shift of g factor from the free electron value. Therefore,

the difference in g factors is the basis for distinguishing various types of thermally generated carbohydrate radicals. The stability of thermally generated radicals depends on the starch structure. Disordering of the starch structure by the process of its oxidation favours radical stabilization. The stability of radicals created in starch oxidized with copper catalyst is controlled also by metal ions, which influence both the process of radical generation, and that of their quenching.

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