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Influence of heating time and pressure treatment of potato starch on the generation of radicals: EPR studies

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ABSTRACT

The influence of heating time and pressurizing time under pressure of 1 GPa on radicals generation in potato starch polymer was investigated by EPR. Potato starch was heated at two temperatures: 413 ± 2 K (140 °C) and 473 ± 2 K (200 °C) for 15, 30, 60, 120, 180, 240, 270 and 300 min and subdued to pressurizing under pressure of 1 ± 0.002 GPa for 6, 60, 300 and 1440 min. In starch heated at 413 K (140 °C) a decrease in the relative intensity of the EPR signal as a function of the pressurizing time can be observed, whereas in starch heated at 473 K (200 °C) such a decrease is not observed. The EPR spectra analysis indicates that they are powder spectra due to the paramagnetic centers leading to Lorentzian line shapes with weak spectroscopic splitting factor g anisotropies and linewidth anisotropies. We have shown that pressurization of starch causes a decrease in the number of radicals in a temperature range in which the polymer is not yet disintegrated. The results of the EPR investigations indicate the existence of a carbon radical center situated at the carbon C1 of the glucose ring. In the EPR spectra of the samples heated with no oxygen access, the hyperfine structure is poorly visible. In the registered EPR spectra of the samples heated with oxygen access, the hyperfine structure is not observed.

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1. Introduction

Radicals in polysaccharides such as potato starch are generated thermally, mechano-chemically and by ionizing irradiation. It is widely accepted that the enumerated factors induce separating of hydrogen atoms from glucose molecules and cleavage of the O–H or C–H bonds. Thermally generated starch radicals are very stable. The localization of the radicals within the starch polymer is not univocally known. Raffi and Agnel (1983) ascertain that unpaired electrons are distributed among crystalline and amorphous regions of the polymer. In another publication, Raffi, Agnel, Thiéry, Frejaville, & Saint-Lèbe (1981) ascertain that crystalline regions of the starch polymer generate radicals of a lifetime longer than the fractions of less ordered structure. Sultankhodzhayeva, Korotchenko, & Yakubova (1983) as well as Thiéry, Agnel, & Battesti (1990) suggest that the radicals in starch originate in the homolytic cleavage of the C–H, C–OH, CO–H bonds. Ciesielski and Tomasik (1996) admit that the unpaired electron is localized on the carbon and/or oxygen atom. Moreover, they assume that the unpaired electron is delocalized, which is confirmed by subsequent theoretical calculations of the electron and spin densities for particular types of radicals by Ciesielski (2007).

Thermal generation of radicals in potato starch has been performed by Dyrek, Łabanowska, & Przetaczek (2007) in two ways: in a microwave oven (800 W, 15 min) or heating in an oven (483 K and 503 K, 30 min). The authors have registered EPR spectra depending on the microwave power in the X-band. The same authors have also registered EPR spectra in the Q-band at constant microwave power for the starch samples previously heated at 483 K for 30 min in the oven. They have demonstrated that the EPR spectrum of potato starch has two components and contains two kinds of radicals. The mechanism of formation of these two components looks as follows. Firstly, the hydrogen atom (Fig. 1) is abstracted from the carbon C1 of the glucose and a type I radical is produced. In the EPR spectrum, a characteristic hump on the left-hand side (low magnetic field) appears. Subsequently, due to the dehydration at the carbons C2 and C3 a type II radical is produced. It has also been noticed that the number of radicals generated decreases with time.

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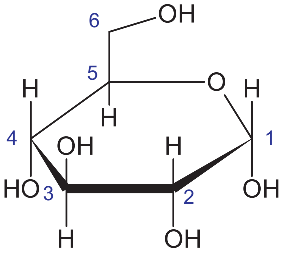

Fig. 1. Structure of glucose unit in starch.

Błaszczak, Dyrek, & Wenda (2010), Dyrek et al. (2007), and Szymońska, Wenda, & Walczak (2010) suggest that this radical type is due to cleavage of the homolytic 1,4-glycoside bond, separating of hydrogen atoms situated at carbon C1 from glucose ring and simultaneous dehydration of the C2 and C3 carbons, which leads to a formation of a double bond.

Formation of carbon radicals is related with the change in hybridization of the carbon C1 from tetrahedral sp^3 into flat sp^2 (Kuzuya, Kondo, & Noguchi, 1992).

Thermally generated starch radicals are very stable. Such stability can be explained as a consequence of the delocalization of unpaired electrons and the steric hindrances (Ciesielski & Tomasik 1996; Szymońska et al., 2010). The type II radicals (showing EPR spectra with no hyperfine structure) are more stable than those of type I (Szymońska et al., 2010). Their higher stability is additionally enhanced by the presence of the unsaturated double bond in the glucose ring (between the carbons C2 and C3).

In starches heated with application of oxidants, the presence of carboxylic and carbonyl groups (Bidzińska, Dyrek, Fortuna, Łabanowska, & Pietrzyk,, 2004) was observed.

In the studies by Kuzuya, Niwa, & Xu (1994) it has been found that molecular oxygen can react with radicals only in one specific site of glucose in amylose. It is testified by the decrease in the number of radicals along with time of exposure to the action of oxygen O_2 in air. Oxygen can associate to a specific radical center in the glucose ring. It is confirmed by high mean values of the spectroscopic splitting factor g_{av} and by only hardly visible hyperfine splitting in the EPR spectrum (Kuzuya, Yamauchi, & Kondo, 1999; Łabanowska, Bidzińska, & Pietrzyk, 2009; Szymońska et al., 2010).

The quantum-chemical calculations: by the AM1 method (Ciesielski, 2007) and by the INDO method (Bernhard, Close, Hüttermann, & Zehner, 1977) indicate very high spin densities at the C–H σ bonds in the oxygen radicals.

On investigating the phosphorylated potato starch (Błaszczak et al., 2010) and in the phosphorylated maize starch (Błaszczak, Dyrek, & Michalec, 2011) the authors have observed both increase in the radical numbers after phosphorylation and decrease after application of high hydrostatic pressure.

Kameya, Nakamura, Ukai, & Shimoyama (2011) ascertained that in γ rays-irradiated starches of various types, EPR spectra show a characteristic doublet. Its presence is explained in terms of interaction between the unpaired electron situated at the carbon C5 of the glucose unit and the proton H5 situated at the carbon C5. This interpretation differs from that proposed by Błaszczak, Dyrek, & Wenda (2008), Dyrek et al. (2007), and Szymońska et al. (2010).

A review on EPR study of thermally generated radicals in starches was given by Bidzińska, Dyrek, & Wenda (2010). The authors have discussed the influence of various factors on paramagnetic centers in starches.

Kudła and Tomasik (1992) when studying potato starch came to the conclusion that the structure of starch under high pressure reveals a dynamic behavior and is dependent on the time of pressurization.

Pei-Ling, Xiao-Song, & Qun, (2010) in a review were stating that hydrostatic high pressure is an effective technology in starch application.

It is well known that starches, including potato starch, in the production conditions do not exhibit the presence of radicals. Application of high pressure without preceding heating does not generate the radicals, too.

The aim of the work is the investigation of the influence of the heating parameters (temperature, time) on the formation of radicals in potato starch and the subsequent investigation of the effect of pressurizing time under high pressure (pressurizing in a hydraulic press) on the amount of radicals. Practical considerations were taken into account when choosing the heating temperatures (food processing: boiling, frying and roasting).

2. Material and methods

The material under investigation was taken in form of dry powder of potato starch from Przedsiębiorstwo Przemysłu Ziemniaczanego S. A., Niechlów, Poland. In the technological process, the potatoes were crumbled, rinsed and refined with water. The grains of potato starch were separated from water suspension in a vacuum filter and dried in a drier to a humidity of 15% of water. The product obtained had the form of white, granular powder. In our procedure of preparation of samples we have not applied high pressure on starch in water-suspension.

Starch was heated in an oven with air access at 413 ± 2 K (140 °C) and 473 ± 2 K (200 °C) in the function of heating time. The two heating temperatures were chosen in order to compare the behavior of the polymer partially decomposed into dextrin under thermal treatment at 473 K (200 °C) and that not yet decomposed at 413 K (140 °C). The thermally treated samples were processed in the presence of air. In order to avoid a longer time of exposure of the samples on air oxygen, the EPR measurements were provided immediately after heating.

High pressure in our experiments was applied on samples in which radicals were generated by thermal heating in oven. In our study we have not observed any generation of radicals only after high pressure treatment without preceded heating. We have applied high pressure to each heated sample after different heating times. The aim of our work was the investigation of the influence of high pressure on the concentration of radicals previously thermally generated by heating in an oven. In Fig. 2 the construction schema of hydraulic press used in pressurization procedure is presented. The material was pressurized under hydrostatic pressure of 1 ± 0.002 GPa in the hydraulic press for 6, 60, 300 and 1440 min. The pressurization procedure was conducted in the presence of air. We suppose that the exposure of the samples to air during pressurization at room temperature had a small influence on the concentration of radicals.

The color (*Lab* color space) of the potato starch with 15% water content was denoted according to the $L^*a^*b^*$ Color System (International Commission on Illumination – CIE 1976) with the Minolta Chroma Meter CR-300, Japan. The results are given in Table 1. The content of particular components in potato starch as related to the mass of dry product is: the ash content amounts to 0.28 weight %, protein content – 0.08 weight %, fat – 0.05 weight %. Fig. 3 shows the statistical distribution of the grains dimensions of the potato starch determined with the Fritsch Particle Sizer Autosieb/A20 apparatus. The solid state density of the starch under investigation amounts to 1.480 g/cm^3.

The samples were investigated at room temperatures with an EPR spectrometer type SE/X-2543 manufactured by RADIOPAN, Poznań, Poland, and an EPR spectrometer type ELEXSYS 500 manufactured by BRUKER, Karlsruhe, Germany, in a standard chamber,

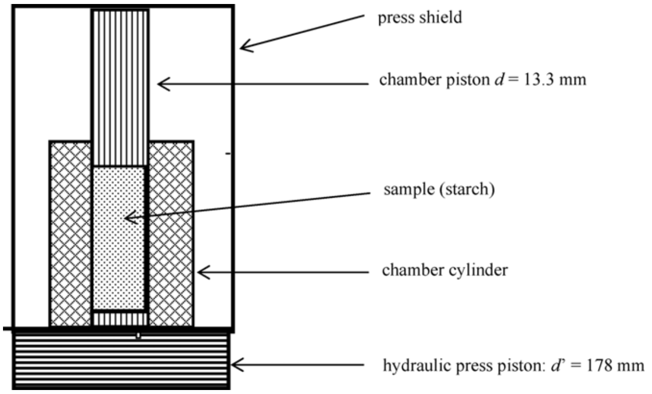

Fig. 2. Construction schema of hydraulic press used in pressurization procedure. Pressurization of potato starch was performed under pressure $p = 1$ GPa in a cylindrical chamber with diameter $d = 13.3$ mm using the hydraulic press with diameter of the piston $d' = 178$ mm made from a hardened steel. The hydrostatic pressure p' applied to hydraulic press piston was calculated from the formula: $p' = p(d/d')^2$, where p' is the applied hydraulic press piston pressure to obtain the desired pressure in the chamber, p is the desired pressure in cylindrical chamber with potato starch sample, d the diameter of chamber piston, d' the diameter of hydraulic press piston.

Table 1
Indication of color space of natural potato starch (15% moisture) – based on light reflection of the surface of sample according to the $L^*a^*b^*$ Color System (CIE 1976), where L is a measure of lightness, and a, b are the color-opponent dimensions (Minolta Chroma Meter CR-300, Japan).

Drying	Calcination	L	a	b
Undried	Uncalcinated	92.74	−1.30	+3.42
120 °C, 1 h	120 °C, 1 h	92.78	−1.39	+4.26
120 °C, 1 h	140 °C, 1 h	92.41	−1.37	+4.71
120 °C, 1 h	160 °C, 1 h	92.27	−1.22	+6.00
120 °C, 1 h	180 °C, 1 h	90.83	−0.99	+8.55
120 °C, 1 h	200 °C, 1 h	75.34	+3.85	+23.12
120 °C, 1 h	220 °C, 1 h	58.00	+7.74	+23.79

in the X-band (9.4 GHz) with magnetic field modulation of 100 kHz and a modulation amplitude of 0.1 mT. Ruby crystal served as a standard of the EPR relative intensity.

The analysis of the EPR spectra was performed by computer simulation assuming Gaussian or Lorentzian shape of individual lines.

The relative intensity of EPR signal was calculated by comparison of the line intensity of the sample being under investigation and the line intensity of the ruby crystal.

The absolute values of the EPR parameters and their accuracy were calculated by using a computer program.

3. Results

We are interested in the behavior of the natural potato starch polymer as a function of heating time. Potato starch of natural 15–19% water content was used. For investigations of time dependence two temperatures were chosen: temperature 413 K (140 °C) at which the polymer is not decomposed and temperature 473 K (200 °C) at which the polymer is gradually decomposed into dextrin, until it is completely decomposed (carbonized). Fig. 4 shows the registered EPR spectra of non-pressurized starch heated in an oven at 413 K (140 °C) (lower curve) and at 473 K (200 °C) (upper curve) for 60 min.

The potato starch samples were heated at 413 K (140 °C) for 30, 60, 90, 120, 180, 240 and 300 min. The color of the compound did not vary with the heating time which testified the absence of decomposition processes at that temperature. The structure of the polymer is preserved. Isotropic Gaussian and/or Lorentzian lines and their combinations do not lead to good fittings to EPR spectra. The analysis of the EPR spectra showed that they represent well the powder spectra with anisotropic g-factor fitted by Lorentzian line shapes. In Fig. 5 the relative intensity of the EPR signal measured with respect to the ruby standard as a function of heating time is presented. The highest EPR signal intensity was observed after 60 min of heating, with the following gradual decrease and a tendency to attain a stable value. The maximum apparent in Fig. 5 is probably related with water evaporation.

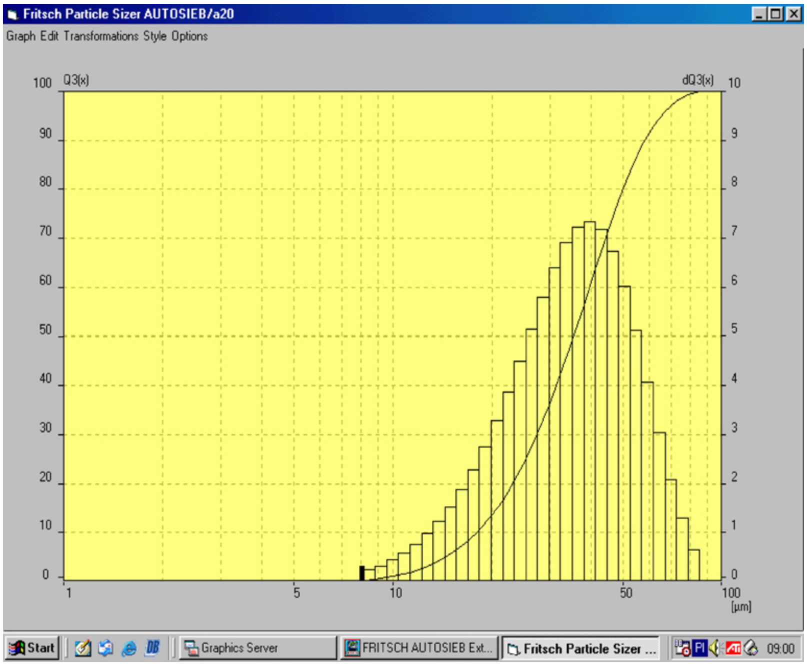

Fig. 3. Statistical distribution of grains dimensions of potato starch found with a Fritsch Particle Sizer Autosieb/A20 apparatus.

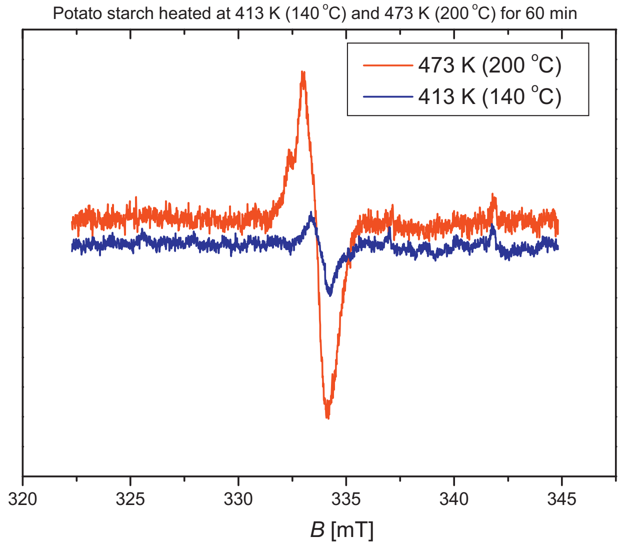

Fig. 4. Comparison of EPR spectra of potato starch heated at 413 ± 2 K (140 °C) and 473 ± 2 K (200 °C) for 60 min at ambient pressure (the differences in shift of the lines are caused by differences of microwave frequencies). The spectra were accumulated 100 times.

Subsequently, the material was heated and pressurized under pressure of 1 GPa in a hydraulic press for 6, 60, 300, 1440 min (Fig. 6a). In Fig. 6a the dependence of the relative EPR signal intensity as a function of the pressurizing time is shown. The highest line in Fig. 6a concerns the non-pressurized material. From Fig. 6a we can see that the application of pressure leads to a lowering of the relative EPR signal intensity, which corresponds with the lowering of the number of radicals. The diagram shows the influence of the time of pressurization on the decrease in radical concentration in the polymer. We postulate a recombination mechanism responsible for the decrease in the number of radicals in polymer under influence of pressure. In Fig. 7 the dependence of the g_z factor of a sample heated at 413 K (140 °C) for 60 min as a function of pressurizing time in a hydraulic press under pressure of 1 GPa is shown. During the pressurizing process, the value of the g_z factor of the sample decreases and after pressurizing time of 300 min amounts to about 2.006. This testifies a decrease in the concentration of oxygen radicals and an increase in the concentration of carbon radicals with pressurizing time of the sample.

Starch with natural water content (15–19%) was heated also in an oven at a temperature of 473 K (200 °C). The registered EPR

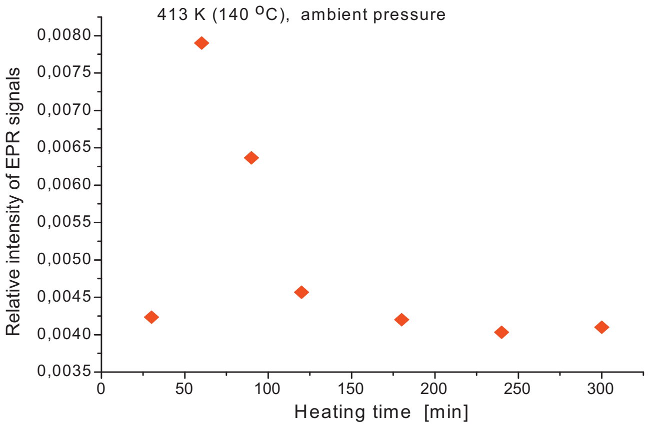

Fig. 5. Relative intensity of EPR signal of potato starch heated at 413 ± 2 K (140 °C) as a function of heating time. Ruby applied as intensity standard. The relative error in determining the relative intensity is about 0.12 (12%).

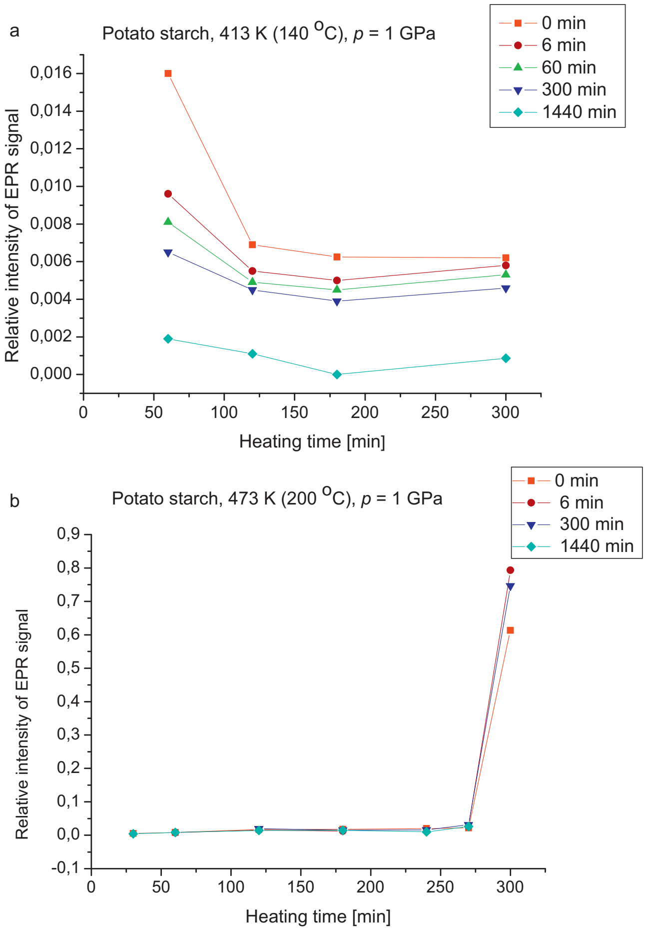

Fig. 6. Relative intensity of EPR signal of potato starch pressurized under pressure $p = 1 \pm 0.002$ GPa in a hydrostatic press as a function of heating time. (a) Heating temperature $T = 413 \pm 2$ K (140 °C), pressing time $t = 0, 6, 60, 300, 1440$ min; (b) heating temperature $T = 473 \pm 2$ K (200 °C), pressing time $t = 0, 6, 300, 1440$ min. Ruby applied as intensity standard. The relative error in determining the relative intensity is about 0.06 (6%).

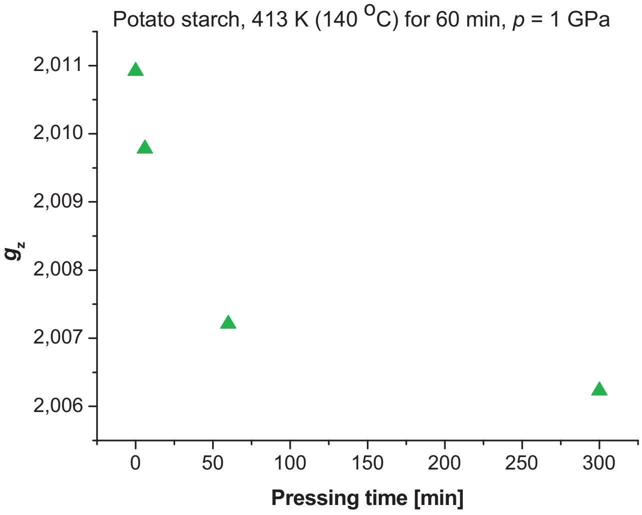

Fig. 7. Changes in the g_z factor of potato starch heated at 413 ± 2 K (140 °C) for 60 min under pressure $p = 1 \pm 0.002$ GPa as a function of pressing time; the g_z-factor was calculated with a fitting procedure. The errors were estimated by the fitting procedure as (pressing time, g_z-factor) = (0 min, 2.0109 ± 0.0008), (6 min, 2.0098 ± 0.0010), (60 min, 2.0072 ± 0.0016), (300 min, 2.0062 ± 0.0044).

spectrum is shown in Fig. 4 (upper curve). A progressing variation in polymer color, from white after 15 min of heating to creamy, yellow and brown during the heating time up to the total carbonization after 300 min of heating in the oven was observed. The change in color suggests a gradual disintegration of the polymer structure during heating time up to complete disintegration after 300 min of heating. Heating at temperature exceeding 473 K (200 °C) was not performed on account of the progressing polymer disintegration. In the samples the presence of a low amount of the type I radicals with hyperfine structure is apparent. It is testified by a hump apparent on the left-hand (low field) side of the EPR spectra (Fig. 4). A similar hump has also been observed by Szymońska et al. (2010) (p. 181). An analysis of the EPR spectra obtained at 473 K (200 °C) indicates that the EPR spectrum represents also the powder spectrum for paramagnetic centers with an anisotropy of the *g* factor on the assumption of a Lorentzian line shape, which additionally indicates the presence of dipolar and exchange interactions between the unpaired electrons. The intensity of the EPR signal increases gradually along with the progress of heating and after 300 min a rapid growth in the EPR signal intensity of the carbonized material becomes visible. The relative EPR signal suggests a completely different behavior of the polymer heated at 473 K (200 °C) (Fig. 6b) with heating time with respect to the polymer heated at 413 K (140 °C) (Fig. 6a). The characteristic maximum testifying for water evaporation, like that observed at 413 K (140 °C) after 60 min heating, was not observed in the case of heating at 473 K (200 °C). At 473 K (200 °C), water evaporated very quickly, probably completely after 5–10 min of heating. Fig. 8a shows a photograph taken with an optical microscope at about 1000-fold magnification of the polymer structure after 270 min heating at 473 K (200 °C) with a yet conserved grain structure; Fig. 8b shows the completely carbonized polymer after 300 min of heating at 473 K (200 °C).

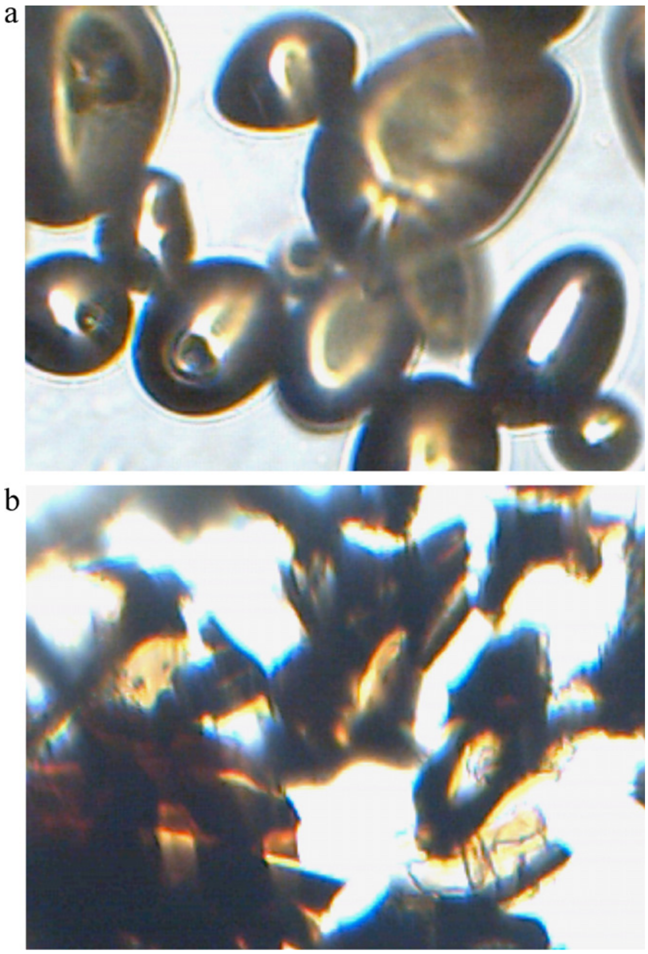

Fig. 8. Picture taken with an optical microscope with about 1000-fold magnification of the structure of potato starch polymer heated at 473 ± 2 K (200 °C); (a) after 270 min of heating, with preserved grain structure; (b) after 300 min of heating, with structure destroyed by carbonization.

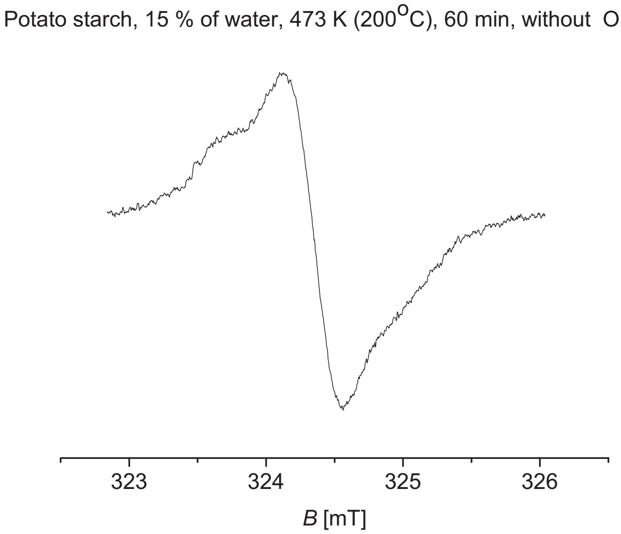

Fig. 9. Exemplary EPR spectrum of potato starch heated at 473 ± 2 K (200 °C) for 60 min without oxygen O_2 access. Additional two side lines represent the hyperfine structure due to the interaction of the unpaired electron with one hydrogen ^1H nucleus.

The material heated at 473 K (200 °C) for different times (from 15 to 300 min) was pressurized under hydrostatic pressure of 1 GPa in a hydraulic press for 6, 300 and 1440 min. Fig. 6b shows the dependence of the relative EPR signal intensity as a function of heating time for these samples. As it can be seen in Fig. 6b, in the case of a polymer partially decomposed into dextrin (heated at 473 K (200 °C)), no influence of the pressurizing time on the decrease in the radical concentration is observed. It follows that a recombination of radicals is absent.

The potato starch material was also heated in a oven at 473 K (200 °C) with no oxygen access for 60, 120 and 180 min. In the samples heated, the carbonization process occurred. In the samples heated with no oxygen access, the presence of a low amount of the type I radicals with hyperfine structure is also apparent (Fig. 9). The analysis of the EPR spectra indicates that these are also powder spectra, originating from paramagnetic centers, with Lorentzian line shapes with a low anisotropy of the *g* factor and linewidth anisotropy.

4. Discussion of results

The shape of the EPR spectra in all our measurements indicates that type II radical (95%) is involved, which is schematically shown in Fig. 10.

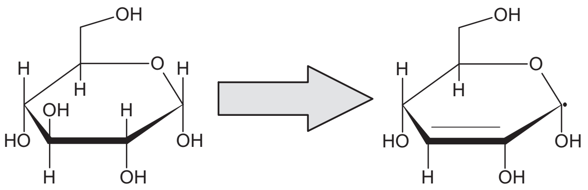

Fig. 10. Scheme of the change in the structure: glucose unit to type II carbon radical (according to Dyrek et al., 2007).

In the samples heated with the access of air oxygen at 413 K (140 °C) as well as at 473 K (200 °C)), the value of the g_z factor exceeding 2.006 was found (Fig. 7), which testifies a greater role played by unpaired electrons localized at oxygen atom (oxygen builds itself into the polymer structure). This indicates a stronger affinity of the oxygen O to unpaired electron localized at the carbon C1 atom in glucose. Schematically, it can be presented as follows:

$$R\cdot + \cdot O\cdot \rightarrow R\text{–}O,$$

where R denotes the glucose ring with a carbon radical center situated at the C1 carbon.

The EPR spectra obtained are complex, formed of unseparated signals (originating in the powder spectrum if the Lorentzian lineshapes are assumed) that cannot be attributed to particular radicals situated at carbon or oxygen atoms. This fact serves as a basis of the assumption on the unpaired electron delocalization. The presence of a Lorentzian line in the EPR spectrum indicates the occurrence of the dipolar and exchange interactions between the unpaired electrons. The low anisotropy of spectroscopic splitting factor g indicates that a carbon radical center is placed at the carbon C1 of the glucose ring (Kuzuya et al., 1992, 1994). The obtained values of the spectroscopic splitting factor g exceeding 2.005 indicate that oxygen from air joins the carbon radical center at the carbon C1 (Kuzuya et al., 1994, 1999).

The molecular oxygen can react with radicals at only one place of glucose: in amylose as confirmed by a decrease in the number of radicals with heating time related with a longer exposition to air oxygen. A higher value of the mean g_z factor exceeding 2.006 and imperceptible hyperfine structure in our EPR spectra can be explained in terms of association of two air oxygen atoms to the carbon radical center localized at the C1 atom of glucose unit. This causes a cleavage of the bond between the carbon C2 and H in the glucose ring. The reaction of carbon radicals (at C1) with oxygen leads to a change in the carbon hybridization from a flat sp^2 one into the tetrahedral sp^3 one (Kuzuya et al., 1994).

A longer heating time or pressurization leads to a reduced value of g_z and to that of the mean g_{av} value, which indicates a lowering of the action of the oxygen atom on the radical center localized at the carbon C1. It can be related with the cleavage of the bonds between oxygen and carbon radical center as well as of the hydrogen bonds in the polymer.

The higher is the value of the coefficient g_z, the higher is the amount of oxygen in the polymer (localization of the unpaired electron on the oxygen atom), which is a consequence of the fact that the unpaired electron occupies the nonbonding p_z orbitals of the oxygen atom due to high value of the spin–orbit coupling constant in the oxygen atom. A transition of the electron from an orbital with one paired electron pair to an unpaired orbital leads to a significant enhancement of the g-factor. Earlier it has been found in radicals of the alkoxyl type arising due to x-ray irradiation in crystals of nucleotides and nucleosides (Bernhard et al., 1977).

From decreasing value of the g_z factor it can be deduced that the oxygen radicals gradually transform in time into carbon radicals (Fig. 7). It is beyond doubt related with a lower value of the spin–orbit coupling constant for carbon. As seen in Fig. 7, the value of the g_z factor of the non-pressurized samples amounts to 2.011, which testifies for a larger contribution of oxygen radicals (due to a high value of the spin–orbit coupling constant in oxygen atom).

Błaszczak et al. (2008) investigated the influence of pressure of 650 MPa acting for 9 min on the decrease in the radical concentration in corn starch as compared with non-pressurized samples. After pressurization performed on aqueous suspension of the starch (30% of H_2O) water was removed by drying in vacuo and then the solid residue was heated in an oven for 30 min at 453 K (180 °C), 483 K (210 °C) and 503 K (230 °C). They found a distinct influence of pressure on the decrease in the number of radicals in the sample. In their opinion it is due to water penetration into polymer grains. Similar dependences were observed in our study of potato starch heated at 413 K (140 °C) (Fig. 6a). The main idea of our experiment is different from that one of Błaszczak et al. (2008). In their experiments the pressure procedure was applied before thermal heating and it was used to prepare the samples. In our experiment the pressure procedure was applied after thermal heating of the samples. We have shown that the effect of the time of pressurization procedure causes lowering the number of radicals generated previously by heating.

In their simulations of the EPR spectra, Ciesielski and Tomasik (1996) admit the possibility of occurrence of 12 radical types: six types originating from carbon atoms and six from oxygen atoms. In all the simulated radical types, intermolecular interactions between unpaired electrons were admitted with exception of carbon radical at carbon C4 in glucose, for which no such interactions were allowed. Experimentally, only the first type of the simulated EPR spectrum on the assumption of intermolecular spin–spin interactions was observed. On the basis of our EPR spectra it can be admitted that intermolecular interactions between unpaired electrons are present. If there were no such interaction, the EPR spectrum should, according to the simulations, show a clear hyperfine splitting (Ciesielski and Tomasik, 1996, Fig. 4, p. 209). However, this is not observed in our spectra. The EPR spectra registered in different laboratories for potato starch (and other kinds of starch) univocally indicate that in starch polymer intermolecular interactions between unpaired electrons are always present.

Tomasik, Bidzińska, & Dyrek (2008) performed an analysis of the EPR spectra of radicals generated by thermal treatment and by x-ray irradiation in corn starch dextrin. They found that the shape of the EPR spectra varies from mostly Lorentzian after heating for 30 min at 503 K (230 °C) to a mixture of Lorentzian and Gaussian lines after heating for 70 min at 573 K (300 °C) with the increase in the heating temperature. The authors ascertained that a gradual vanishing of the Lorentzian character of the EPR signal indicates a gradual vanishing of the exchange interaction between radicals. An analysis of our EPR spectra at 413 K (140 °C) as well as at 473 K (200 °C) indicates a Lorentzian character of the spectra, which suggests the presence of intermolecular (dipolar and exchange) interactions between unpaired electrons.

At temperatures above 433 K (160 °C), starch is subdue to a gradual dextrinization process and above 473 K (200 °C) this process runs violently. The starch dextrinization consists in a cleavage of the polysaccharide chain into smaller fragments called dextrins. The degree of dextrinization corresponds with the degree of starch chain destruction. The higher it is the more the natural properties of starch are disturbed. A question can be raised if the starch polymer heated at a temperature above 473 K (200°C) does preserve its structure? Beyond doubt, a partial destruction of the polymer structure can occur, but as long as there is no carbonization, the grain structure of the polymer is preserved despite the change in color from creamy to brown with the course of heating time. In current work, the samples heated with access to oxygen from air at 473 K (200 °C) for 270 min preserved their grain structure (Fig. 8a) and those heated for 300 min were characterized by an appearance of black color testifying for carbonization and simultaneous destruction of polymer structure (Fig. 8b). At the temperature of 473 K (200 °C) a total vanishing of the grain structure of polymer occurred.

For the sake of comparison note that samples heated at 413 K (140 °C) for 30–300 min do not show any changes in color. At this temperature no essential changes in the polymer structure were observed. The highest relative EPR signal intensity corresponding to the amount of radicals generated at a temperature of 413 K (140 °C) is observed after 60 min and, subsequently, decreases in further heating. In that critical time (60 min, Fig. 5) a complete dehydration of the starch occurs with simultaneous appearance of maximal number of radicals.

5. Conclusions

- Heating of starch at 413 K (140 °C) for 30–300 min does not lead to a destruction of starch polymer. After 60 min of heating, a characteristic maximum in the relative intensity of EPR signal appears which can testify for the end of the water evaporation process.
- Radicals content in starch heated at 413 K (140 °C) and subsequently pressurized under pressure of 1 GPa gradually decreases with pressurizing time (60–1440 min).
- Heating at 473 K (200 °C) causes a slow increase in the relative EPR signal intensity during heating which is accompanied by partial dextrinization of the starch polymer. After 300 min of heating an abrupt signal increase follows, marking the complete polymer destruction due to the carbonization process.
- All registered EPR spectra show contributions from two kinds of radicals: type I radicals with hyperfine structure are relatively low in number and type II radicals without hyperfine structure that prevail in number.
- High hydrostatic pressure 1 GPa acting on previously heated starch lowers the concentration of radicals.

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