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The mechanism of thermal activated radical formation in potato starch studied by electron paramagnetic resonance and Raman spectroscopies

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ABSTRACT

Degradation of starch and the constituent fractions: amylopectin and amylose during thermal treatment in the range 423–503 K was investigated by electron paramagnetic resonance (EPR) and Raman spectroscopy. Degradation process was accompanied by the generation of carbon-centered stable radicals. EPR provided data on the nature and structure of radicals and on their evolution upon thermal treatment, whereas Raman spectroscopy allowed monitoring the changes of bonds in polysaccharides. It was found that amylose was the most susceptible toward high temperatures and the process of radical generation started at lower temperature than in amylopectin and starch, which were more resistant to thermal degradation.

1. Introduction

Potato starch is produced from tubers of potato (*Solanum tuberosum*). It consists mainly of two polymeric fractions: amylose and amylopectin. In comparison with starches from other sources, it is rich in phosphorus (0.09%), present mostly in the form of phosphate monoesters and contains small amounts of lipids (0.19%) and nitrogen (0.1%) (Hoover, 2001).

Amylopectin is the main polymeric fraction of the starch, whereas amylose content is lower and in the potato starch reaches 16.6–25.4% (Hoover, 2001; Noda et al., 2005; Singh, Isono, Srichuwong, Noda, & Nishinari, 2008). Both polysaccharides form chains built of α-D-glucose units. Amylose is almost linear and the glucose units are linked with 1,4-glycosidic bonds. The 1,6-glycosidic linkages, responsible for branching, amount only to 0.1% of bonds (Tegge, 2010). Amylopectin is highly branched and the content of branching bonds may reach 4%. As a result of branching, amylopectin molecule is compact for its high molecular weight. It is built of three types of chains differing in length and the most abundant are the short ones. Its structure is complex and the branching within the molecule is not random. Models usually depict it as a cluster type organization with shorter linear chains arranged in clusters on longer chains (Hoover, 2001; Parker & Ring, 2001). Starch molecules are arranged in granules containing amorphous and crystalline domains. The latter are built mostly of amylopectin (Parker & Ring, 2001; Tegge, 2010).

Potatoes are grown throughout the world being one of the basic crops. The large part of the yield is processed into starch, which is subjected during subsequent treatment to chemical and physical modifications, such as phosphorylation, oxidation, radiation, thermal and mechanical treatment. Changes of physicochemical properties occurring upon such modifications should be recognized because they could have essential implications for food quality and consumers' health. Temperatures in the range of 450–520 K, widely applied in starch treatment processes, can cause the degradation of molecules of polysaccharides. Depolimerisation of starch leads to amorphic pirodextrins, which are shorter length molecules, soluble in water. In temperatures higher than 450 K, 1,6-dehydro-β-D-glucopyranose and other low weight molecules are formed (Ciesielski & Tomasik, 1996; Tegge, 2010). Starch thermal degradation is accompanied by the process of radical formation. It is controlled by the structure of starch and its components, the heating temperature and other parameters, such as the presence of various additives, for example metal ions, sweeteners and also by chemical or physical modifications (Łabanowska et al., 2008, 2009, 2011). Various types of radicals with short and long life time can be created during thermal treatment. As it was found in many studies, the latter ones are generated easily and stabilized by the mono- and polysaccharide molecules (Abagyan & Apresyan, 2002; Kuzuya, Yamauchi, & Kondo, 1999; Łabanowska et al., 2008, 2011; Madden & Bernhard, 1982; Yamaoki, Kimura, & Ohta, 2010; Yamauchi, Sugito, & Kuzuya, 1999). Such stable radicals can be observed with the application of electron paramagnetic resonance

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(EPR) spectroscopy, which provides information about their structures and changes in their contents occurring during processes of their generation and decay. On the other hand, Raman spectroscopy is the method which can monitor the changes of bonds in starch molecules during processes of radical formation.

The aim of our work was to study the mechanism of radical formation upon thermal treatment in starch, amylose and amylopectin, obtained from potato tubers. The temperatures in the range often used in starch product processing were chosen to investigate the mechanism of radical generation. Two spectroscopic methods: EPR and Raman spectroscopy were applied to study the changes occurring in the structure of polysaccharides and character of radicals formed during such treatment. The Raman spectroscopy let us investigate the bonds modification in starch and its fractions, occurring in high temperatures, whereas electron paramagnetic resonance (EPR) spectroscopy provided data on the nature of radicals, their content and the influence of the temperature on the process of radical formation.

2. Materials and methods

2.1. Materials

The potato starch (S4251) and amylose (A0512) produced by Sigma–Aldrich and amylopectin from potato starch produced by Fluka (10118) were used for the study. Additionally, potato starch "Superior Standard" from Luboń (WPPZ Luboń, Poland) was investigated.

2.2. Amylose content

Content of amylose in starch samples was determined spectrophotometrically using spectrophotometer Genesys 10S (Thermo Scientific, USA) according to method described by Morrison and Laignelet (1983).

2.3. Phosphorus content

Total phosphorus content was established according to the Polish norm PN-EN ISO 3946 with the use of spectrophotometer Genesys 10S (Thermo Scientific, USA).

2.4. Thermal treatment

The samples of investigated starch and its constituents (about 100 mg) were placed in the quartz EPR tubes and heated in oven in air for 30 min at 423 K, followed by 30 min at temperature 483 K. Samples of starch and amylopectin were also treated at higher temperature (503 K), whereas amylose was additionally heated at 453 K. Before recording of the EPR spectra, the tubes with the samples were cooled to room temperature and closed with a paraffin membrane.

2.5. EPR measurements

The EPR measurements were performed with an X-band Bruker ELEXSYS 500 spectrometer (Karlsruhe, Germany) with 100 kHz field modulation. The spectra were recorded at 293 K with modulation amplitude of 0.3 mT at microwave power 3.0 mW. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a *g*-factor and quantitative standard. As the latter, the sample containing known number of spins (1.07×10^{15} spins/g) was applied. The maximum error of the quantitative measurements was 0.05×10^{15} spin/g. EPR parameters (*g*-factor value, hyperfine splitting constant *A*, peak-to-peak line width ΔB_{pp}) were found by a simulation procedure, using a program SIM 32 (Spałek, Pietrzyk, & Sojka, 2005). The accuracy of determination of EPR parameters was ±0.0001 for *g* values and 0.05 mT for parameters *A*.

2.6. Raman spectra

The Raman spectra were recorded with a Raman microspectrometer model inVia produced by Renishaw (UK), working in confocal mode. All samples before thermal treatment and thermally treated starch and amylopectin were excited with 785 nm laser line of HP NIR diode laser Renishaw (UK). The FT Raman spectra of untreated and heated at 423 K amylose were collected also by FT-Raman Accessory Spectrometer (based on FTS 6000) made by Bio-Rad (USA), with Ge liquid nitrogen cooled detector. Both amylose samples were excited with 1064 nm line of diode pumped Nd:YAG Spectra Physics laser (USA), because energy of 785 nm excitation line was too high for heated amylose sample. The laser power was kept low enough to ensure that it did not damage the sample.

Measurements were performed with microscope and spectra were taken from the same spot size of each sample. Registrations were repeated five times for each sample.

3. Results and discussion

No EPR signals were observed in the potato starch and in its components before thermal treatment (Fig. 1 – reference lines). The species active in EPR appeared in the samples after heating (Fig. 1). The parameters of recorded signals indicated that paramagnetic species originated from carbon centered radicals and their amounts depended on the kind of polysaccharide. Comparison of radicals amount generated in starch with that in amylose (both reagents obtained from Sigma and heated at 483 K) showed 25 times higher number of spins in amylose (Table 1). On the other hand, lower yields of radicals creation were found in pure amylopectin and in starch from Luboń, containing higher quantity of amylopectin (79.2%) than Sigma starch (73.7%) (Table 1). During the heating all samples darkened, starch and amylopectin became light beige at 483 K, the sample of amylose was beige after treatment at 423 K and turned dark brown upon heating at 483 K. Sample of starch heated at 503 K turned dark beige, whereas color of amylopectin changed slightly.

The intensities of the signals recorded in starch and amylopectin were negligible after thermal treatment at 423 K and increased continuously up to 503 K. Simultaneously the shape of the spectra changed, achieving the most complex character after heating the samples at 483 K and simplified for samples treated at 503 K (Fig. 1a–d). Amylose sample gave EPR spectrum after heating at 423 K, however its complexity was less visible than observed for spectra of amylopectin and starch heated at 483 K. With increasing treatment temperature spectrum of amylose strongly narrowed (Fig. 1e and f).

The simulation procedure revealed that the spectrum of native starch produced by Sigma heated at 483 K consisted of four signals of Gaussian character (Fig. 1a) with parameters gathered in Table 1. Signal I was a doublet, which exhibited the highest contribution to the spectrum, whereas signal II with the lower contribution was split on four lines with similar intensities and different *A* constant values. Its *g*-factor was higher than signal I. Signals III and IV exhibited lower *g* values. The former was a single line, while the latter, consisting of three doublets with various values of splitting, had the smallest contribution to the spectrum.

Spectrum of amylopectin treated at 483 K could be decomposed on two main signals: I and II, with parameters close to those of starch signals with the exception of slightly lower *g* value of signal II (Fig. 1c, Table 1). Signal IV had negligible intensity. The contribution of the signal II was practically the same to the spectra of starch and

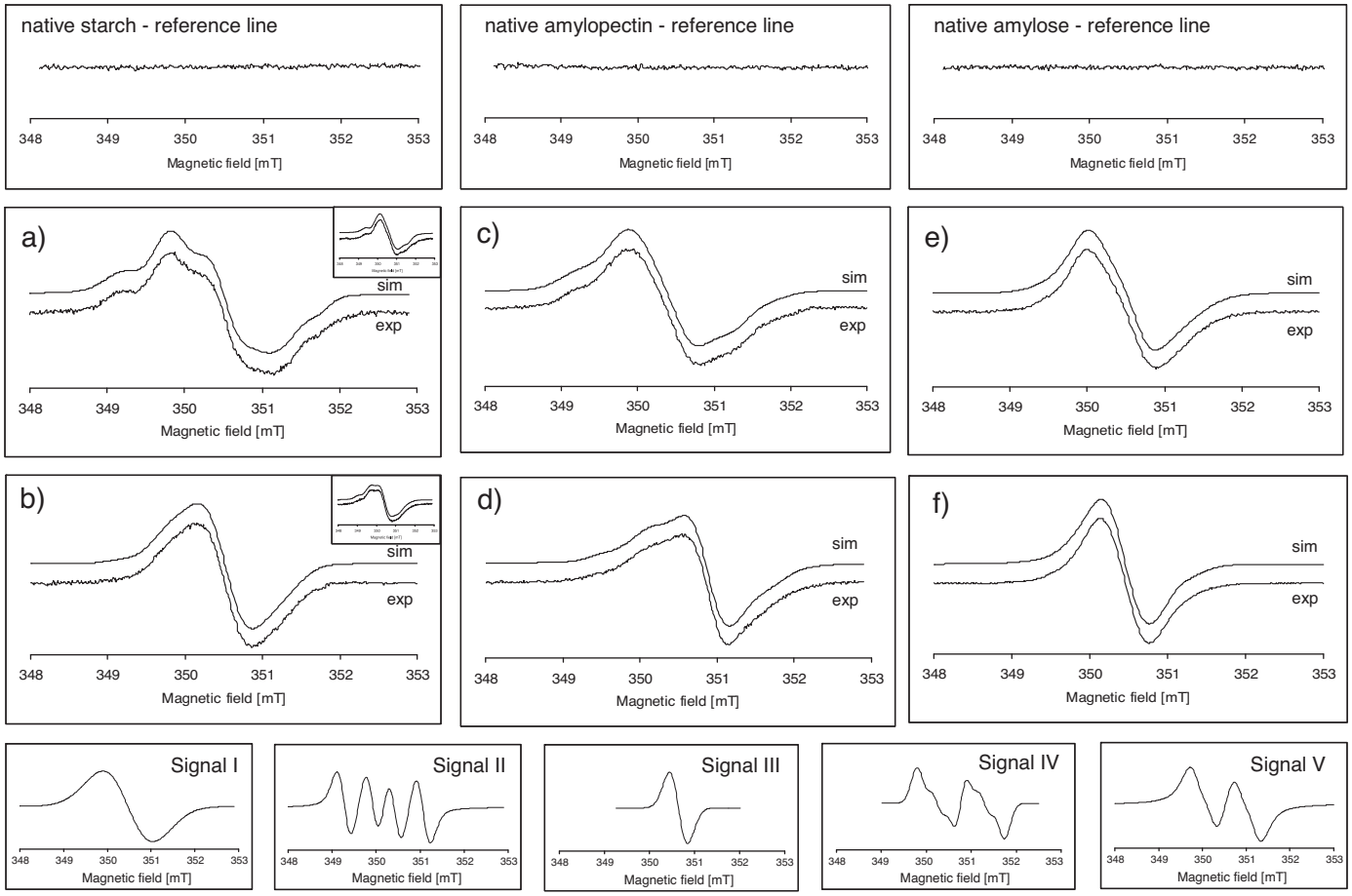

Fig. 1. The experimental and simulated spectra of potato starch and its constituent fractions registered at 293 K in the range of 5 mT, after thermal treatment of the samples. (a) starch (Sigma), insert – starch (Luboń), both thermally treated at 483 K; (b) starch (Sigma), insert – starch (Luboń), both thermally treated at 503 K; (c) amylopectin, thermally treated at 483 K; (d) amylopectin, thermally treated at 503 K; (e) amylose thermally treated at 423 K; and (f) amylose, thermally treated at 483 K. Signals I – V – particular signals used to simulation.

Table 1
EPR parameters of the signals found from simulation procedure in native potato starch and its fractions.

	Temp (K)	Number of spin/1 g	g	A_1 (mT)	A_2 (mT)	A_3 (mT)	Contribution (%)	Signal	Attribution
Native starch (Sigma)	483	4.5×10^{15}	2.0054	0.34			68	I	C(1)
			2.0069	0.64	1.14		10	II	C(6)
			2.0044				16	III	Delocalised[a]
			2.0039	0.40	1.05	0.25	6	IV	C(6)
	503	10.0×10^{15}	2.0050	0.13			22	I	C(1)
			2.0052	0.59	1.10		2	II	C(6)
			2.0040				76	III	Delocalised[a]
Native starch (Luboń)	483	2.0×10^{15}	2.0054	0.49			85	I	C(1)
			2.0069	0.54	1.20		15	II	C(6)
	503	3.5×10^{15}	2.0053	0.40			68	I	C(1)
			2.0069	0.57	1.10		6	II	C(6)
			2.0047				26	III	Delocalised[a]
Amylopectin	483	4.0×10^{15}	2.0053	0.30			85	I	C(1)
			2.0061	0.76	1.14		11	II	C(6)
			2.0034	0.48	0.96	0.18	4	IV	C(6)
	503	9.0×10^{15}	2.0049	0.14			55	I	C(1)
			2.0041				45	III	Delocalised[a]
Amylose	423	49.0×10^{15}	2.0051	0.41			36	I*	C(1)
			2.0043				64	III	Delocalised[a]
	483	102.0×10^{15}	2.0040				88	III	Delocalised[a]
			2.0036	0.32	0.96		12	V	Interacting carbon centers

Where g is the g-factor value; A_1 and A_2 are the values of hyperfine splitting.
[a] Delocalised on conjugated double bonds.

amylopectin. Amylose recorded after thermal treatment at 423 K showed the spectrum consisting of two signals: more intensive signal III, the same as that found in starch spectrum, and signal I* being a doublet with parameters only slightly different than those observed in starch and amylopectin.

With increasing temperature treatment the spectra changed, some signals disappeared and new ones became visible, indicating the transformation of polysaccharide structures (Fig. 1b, d, and f). The most pronounced changes occurred in amylose, where process of degradation started at the lowest temperature (423 K). At this temperature the spectrum of amylose was the closest to spectrum of Sigma starch treated at 503 K (Fig. 1e and b). The changes in the shape of amylose spectrum, occurring upon temperature increase up to 483 K, were accompanied with the vanishing of signal I* and simultaneous increase of signal III intensity, which became the main one in the spectrum. Additionally, low intensive signal V of Lorentzian character, exhibiting low *g*-factor and four lines of hyperfine structure (HFS) appeared. Evolution of the spectra with increasing temperature of thermal treatment was also observed in amylopectin and starch; however, it occurred at higher temperatures (483–503 K). In the case of amylopectin the vanishing of signals II and IV was accompanied with the appearance of new single line with *g*-factor similar to that of signal III. Simultaneously, the contribution of signal I diminished and the values of its *g* and *A* parameter decreased. In starch the changes concerned also all signals, however to a lesser degree than in amylopectin. Signals I and II decreased their contribution, signal IV vanished, whereas contribution of signal III strongly increased at the expense of signal I. The changes in contribution of particular signals to starch spectrum were accompanied with decreasing of their EPR parameters (*g* and *A*) (Table 1). The comparison of the spectra of all investigated samples demonstrated that signal II originated from radicals generated in amylopectin, whereas signal III was formed in both amylose (after heating at 423 K) and amylopectin structure (after heating at 503 K). The main signal I in starch spectrum probably was connected with both fractions: amylopectin and amylose. Although the parameters of signals I* in amylose and I in amylopectin were not quite identical (Table 1), the signals could overlap in the spectrum of starch sample and their resolution in the X-band was not possible. The problem of difficulties in resolution of hyperfine (HF) lines in the spectra of saccharide radicals was already discussed by Vanhaelewyn et al. (2000).

Additionally, it was worthwhile to notice that in the spectrum of starch obtained from Luboń, treated in the same way as that of Sigma production, two main signals (I and II) could be distinguished in the spectrum of sample heated at 483 K (Fig. 1a, insert; Table 1). Further increase of temperature (493 K) resulted in appearance of a signal with *g*-factor similar to signal I, however without HF lines. Its *g*-factor decreased achieving at temperature 503 K value nearing *g*-factor of signal III (Fig. 1b, insert; Table 1). The formation of signal III at higher temperature, similarly as in amylopectin, could be explained by lower amylose content in starch from Luboń (20.8%) than in Sigma starch (26.3%). It was found by Liu, Yu, Liu, Chen, and Li (2009) that decomposition temperature was the lowest for amylose and the highest for amylopectin, hence, starch with lower amylose content (Luboń) was less susceptible to thermal radical creation. Moreover, the presence of phosphorus, incorporated into starch matrix as phosphate groups, decreased the melting enthalpy and increased the amount of thermally generated radicals (Rożnowski et al., unpublished results; Łabanowska et al., paper in preparation) indicating the loosening of starch structure. The phosphorus content almost twice higher in Sigma starch (0.089%) than in starch from Luboń (0.049%) should be also considered as a reason of more effective radical creation in Sigma starch. It was found that solubility and hydration of starch increased with increasing phosphorus content and simultaneously the internal bonds in starch, stabilizing its crystal structure, became weakened (Fortuna, 1994).

It was probable that also other factors (granule size, the origin of starch from different potato cultivars and the year of their harvesting, as well as the details of preparation conditions: oxygen availability during heating in the quartz tubes, local temperature gradients) could influence the process of generation of radical centers.

In order to ascribe particular signals to radicals, we analyzed carefully the spectra recorded for the samples treated at different temperature. The most complex spectra, consisting of four signals originating from amylopectin and amylose fractions were obtained for Sigma starch heated at 483 K.

Signal I could be assigned to the carbon-centered radical formed by abstraction of the hydrogen atom from the carbon atom C(1) of glucose unit. The interaction of magnetic moment of an unpaired electron with nuclear spin of β hydrogen at C(2) led to the signal split on two hyperfine lines. This signal exhibited the similar parameters for starch and amylopectin heated at 483 K and its contribution was higher to the spectrum of amylopectin. In the spectrum obtained after heating of amylose at 423 K, signal I* exhibited only slightly lower value of *g*-factor and similar *A* parameter, therefore we assumed that it could be also attributed to C(1) radical. The similar decrease of *g*-factor value of this signal was observed upon increasing thermal treatment temperature of starch and amylopectin. Such decrease of *g* value could be an indication of the weakening of oxygen atom influence on an unpaired electron localized at adjacent carbon atom. As it was found (Pauwels, Van Speybroeck, & Waroquier, 2006), proximity of electronegative oxygen led to the delocalization of the unpaired electron density from carbon to oxygen atom, which could cause the increase of *g* parameter. Opposite effect suggested the withdrawal of oxygen from carbon atom surroundings, which was probably connected with weakening of bonds, particularly 1,4-glycosidic bond. Simultaneously, diminishing of HFS splitting value, which was accompanied with the decrease of *g*-factor, suggested that also C—H bonds lengthened. The decrease of signal I contribution to the spectra of starch and amylopectin heated at 503 K and its disappearance after thermal treatment at 483 K from amylose spectrum could be an indication of the subsequent degradation of polysaccharide structure.

The signal originating from center II consisted of four lines with two different values of hyperfine splitting, what suggested the interaction of magnetic moment of an unpaired electron with magnetic moments of two non-equivalent hydrogen nuclei. Such radical could be formed by abstraction of the hydrogen atom from C(6) in glucose unit of starch. Then, magnetic moment of an unpaired electron created at C(6) could interact with α hydrogen at C(6) and β hydrogen at C(5). The same radical model was assumed for species generated during thermal treatment in native and oxidized potato starch by Łabanowska et al. (2011) and it was earlier postulated for thermally treated and X-ray irradiated α-D-glucose single crystals by Madden and Bernhard (1979, 1982). The characteristic feature of this signal was its slightly higher value of *g*-factor found in starch spectrum in comparison with amylopectin. It could indicate the greater influence of oxygen atom on an unpaired electron at C(6), resulting from shortening C(6)—O distance in amylopectin fraction forming starch structure. Such reorganization leading to greater proximity of oxygen atom to C(6) could be caused by weakening or even breaking of 1,6-glycosidic bond caused by the presence of amylose component in starch. Values of parameter *A* for HFS of species created in starch and amylopectin were lower than those found for radicals generated in single crystal of glucose (Madden & Bernhard, 1979, 1982), indicating stronger deformation of polysaccharide structures, which occurred during thermal treatment at higher temperature.

Signal III was a single line, whose *g*-factor and intensity mostly depended on the kind of polysaccharide and temperature of thermal treatment. The absence of HF lines pointed to the lack of interactions between magnetic moments of an unpaired electron and hydrogen nuclei, which was probably caused by the removal of hydrogen atoms from polysaccharide molecule, for example in dehydration process, which occurred during heating. With increasing temperature (493 K) the signal with *g*-factor similar to that of signal I, however without HFS, appeared in the spectrum of Luboń starch with simultaneous decrease of the signal I intensity. We assumed that this center was formed from center I. In order to explain its formation, we proposed the following mechanism: first the glucose unit with radical localized at C(1) was dehydrated, so the β hydrogen atom forming water molecule with OH group situated at C(3) was removed and the double bond between C(2) and C(3) was created. Further increase of temperature (up to 503 K) led to decrease of *g* value of this signal which became close to that of signal III, found in the spectrum of Sigma starch heated at 483 K (Table 1). The decrease of *g*-factor value with growing temperature (503 K) was observed also for signal III. It was accompanied with increase of its intensity at the expense of the signal I. In amylose after thermal treatment at 483 K, when sample strongly darkened, signal III became the main signal in the spectrum and its *g*-factor achieved the lowest value, the same as was observed in spectra of Sigma starch and amylopectin heated at 503 K. It suggested the strong diminishing influence of oxygen on an unpaired electron and indicated that the radical structures became depleted in oxygen, what resulted from the gradual dehydration process of polysaccharides occurring in higher temperature. In a consequence, the formation of double carbon–carbon bonds in glucose ring could be postulated. Taking into account the simultaneous lack of HFS, we ascribed signal III to unpaired electron delocalised on conjugated double bonds.

Signal V appearing besides signal III in the spectrum of amylose heated to 483 K, exhibiting Lorentzian shape, could indicate the increase of interactions between radical centers, suggesting the formation of carbonaceous like material.

The low *g*-factor value of signal IV suggested that also this center was located on a carbon atom not adjacent to any oxygen atoms. Moreover, the presence of three doublets of hyperfine structure indicated interaction with three hydrogen atoms. Therefore, we assumed that such radical was created by abstraction of OH group from C(6). In such center the magnetic moment of unpaired electron was coupled with magnetic moments of two α hydrogen atoms bonded to C(6) and β hydrogen at C(5). The same situation would occur, if OH group was abstracted from C(2) or C(3). The distinguishing between models could be achieved by theoretical calculation (Pauwels et al., 2006).

Comparing EPR parameters of radical centers created during thermal treatment of starch and its fractions and following their evolution with increasing temperature it could be noticed that amylose structure was the least stable and the process of its degradation was the most advanced and occurred with the formation of the highest number of radicals (Table 1). In the case of amylopectin and starch thermal radical generation was slower and the changes in EPR parameters of signals, as well as the amount of created radicals were comparable (Table 1).

The Raman spectra of amylose, amylopectin and potato starch before thermal treatment were shown in Fig. 2a and b. It was visible that positions of observed bands were almost the same, but their intensities differed. The intensity of amylose spectrum was lower than those of amylopectin and starch, which probably resulted from its amorphous character. The direct comparison of spectra intensities was possible because the layer thickness and spot laser size were practically identical for all samples. Some differences in the spectra were seen only in rather poorly resolved

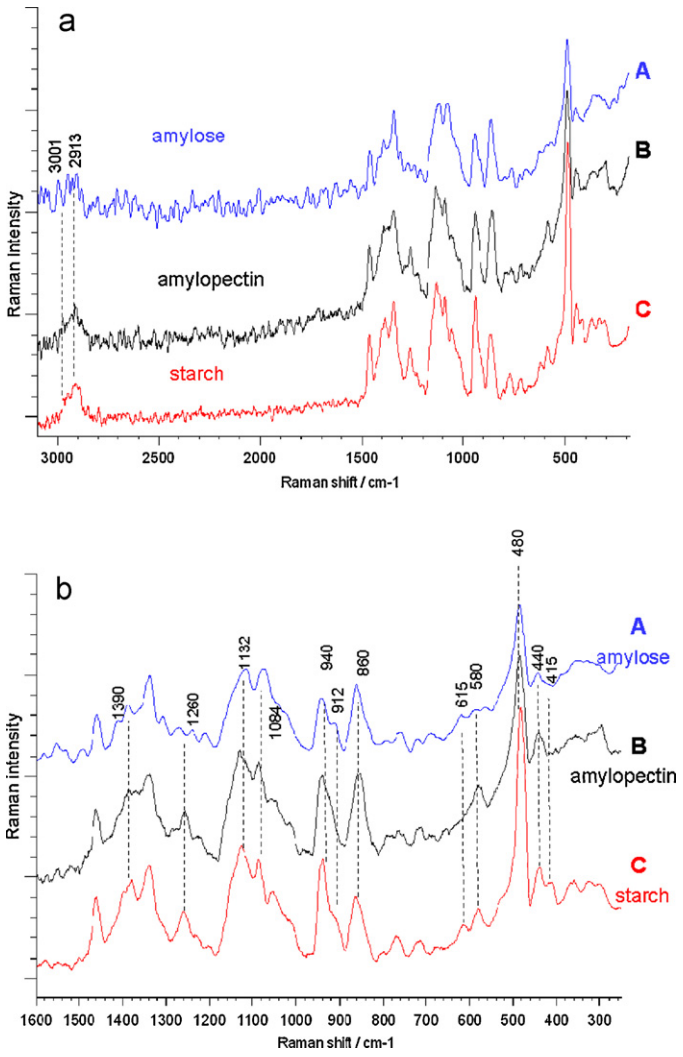

Fig. 2. The Raman spectra of native amylose (A), amylopectin (B) and potato starch (C) (785 nm excitation): (a) in the range 3100–200 cm^{-1} and (b) in the range 1600–250 cm^{-1}.

region at ca. 3000 cm^{-1}, which was O—H and C—H stretching region. The interpretation of the region below 1700 cm^{-1} provided valuable information, as it allowed following changes which occurred in polysaccharide structures upon thermal treatment.

Taking into account the glucose ring as a building unit of polysaccharide chains, it was expected that most of the observed modes in starch and its fractions spectra could be similar to those observed for the glucose molecule (Cael, Koenig, & Blackwell, 1973). Spectra were dominated by a strong band at 480 cm^{-1} due to skeletal mode involving (C—O—C) ring mode and δ(C—C—O). Among other bands (Table 2) the characteristic for polysaccharides were the bands: 860 cm^{-1} originating from ν_s(C—O—C) ring mode and C1—H bending α-configuration, 940 cm^{-1} assigned to ν_s(C—O—C) α-1,4-glycosidic linkage, 1060 cm^{-1} from δ(C—OH) and ν(C—OH), 1145 cm^{-1} due to ν_a(C—O—C) α-1,4-glycosidic linkage and finally 1390 cm^{-1} due to δ(C—H), CH bending and CH scissoring vibrations. The observed bands at about 1460 cm^{-1} could be attributed to $\delta(CH_2)$ twisting and CH bending, whereas 1340 cm^{-1} to $\delta(CH_2)$ and C—OH bending (Table 2; Figs. 2b, 4b and 5b) (Cael et al., 1973; Colthup, Wiberley, & Daly, 1964; Kizil, Irudayaraj, & Seetharaman, 2002; Passauer, Bender, & Fischer, 2010; Sekkal, Dincq, Legrand, & Huvenne, 1995; Soderholm, Roos, Meinander, & Hotokka, 1999).

Table 2
Positions (cm^{-1}) and assignments of Raman bands of potato starch and its fractions before and after thermal treatment.

Amylose native	Amylose treated at 423 K	Amylopectin native	Amylopectin treated at 483 K	Starch native	Starch treated at 483 K	Assignment
3002 (2993)	(2998)					ν(C—H)[e]
2950 (2962)	(2962)	2980	2977	2973		$\nu_{as}(CH_2)$[e]
				2950		
2910 (2915)	(2928) (2915)	2933	2933	2914	2915	$\nu_{as}(CH_2)$[e]
		2914	2913			
2885	(2898) (2893)	2885	2885			$\nu_s(CH_2)$, $\nu_s(CH_3)$[e]
1460	(1462)	1463		1463	1463	$\delta(CH_2)$ twisting, CH bending[b,c]
1416				1401		δ(O—C—H), δ(C—C—H)[c,d]
1390	(1392)	1388	1386	1383		δ(C—H), CH bending[a], CH scissoring
1364						δ(C—C—H)[d]
1337 (1349)	(1349)	1340	1342	1340		$\delta(CH_2)$, C—OH bending[b,c]
1308	(1317)					δ(C—C—H)[c]
(1255)	(1255)	1257	1258	1261	1261	$\delta(CH_2)$, CH_2OH (side chain) related mode[a]
1213						δ(C—H)[b], δ(C—O—H)[d]
1145	(1167)	1158 sh		1150 sh		ν_a(C—O—C) α-1,4-glycosidic linkage[e]
1128	(1132)	1133	1132	1128	1123	ν(C—OH) bending, ν(C—O), δ(C—OH)[e]
1113 (1117)	(1117)					
1074		1087		1087		ν(C—O—C) ring mode, C—OH bending[b,c]
1044		1058		1056	1054	δ(C—OH), ν(C—OH)[e]
		1015				CH_2 related mode
944		941	942	941	940	ν_s(C—O—C) α-1,4-glycosidic linkage[a]
911		924		911	914	α-configuration C—OH bending[b,c,d]
860		855	854	862	862	ν_s(C—O—C) ring mode, C1—H bending α-configuration[e]
840						$\delta(CH_2)$, ν(C–C), ν(C—O)[c]
758				769		ν(C—O)[a]
579		578	579	578		skeletal modes[e]
483 (481)	(481)	483	482	480	481	skeletal mode involving (C—O—C) ring mode, δ(C—C—O)[e]
443		440	440	440		δ(C—C—C)[d]
418				411	414	δ(C—C—O)[d]

Samples of amylopectin, starch and native amylose were excited with 785 nm laser line, samples of amylose native and thermally treated were excited also with 1064 nm line (data in brackets).

[a] Cael et al. (1973).
[b] Passauer et al. (2010).
[c] Sekkal et al. (1995).
[d] Soderholm et al. (1999).
[e] Colthup et al. (1964).

The detailed analysis of Raman spectra revealed the differences in intensities of particular bands in starch and its fractions. The most differentiating was the band at 940 cm^{-1} originating from ν_s(C—O—C) α-1,4-glycosidic linkage, which was more intensive in amylopectin and in consequence in starch, than in amylose, confirming better long-range order in structures of the former polysaccarides (Fig. 2b).

The degradation process of amylose, amylopectin and potato starch induced by heating, described in experimental section, was monitored by Raman spectroscopy. During thermal treatment at 483 K amylose sample, as it was mentioned before, underwent the most considerable destruction, changing from white powder toward dark-brown material of the consistency like cotton wool. Fluorescence of the sample was strong; consequently it was not possible to detect any Raman bands. Therefore, amylose sample was analyzed after treatment at lower temperature (423 K) at which its color turned slightly beige. Moreover, since energy of 785 nm excitation line was too high, the 1064 nm line was chosen to collect the actually very weak spectrum of almost disintegrated sample. Spectra of native and thermally treated amylose excited with 1064 nm line were shown in Fig. 3, whereas Fig. 2 presents spectrum of unheated amylose excited with 785 nm line, similarly as amylopectin and starch.

The comparison of the spectra of native and thermally treated amylose (Fig. 3) showed total vanishing of its cyclic structure (vanishing of band at 480 cm^{-1}). Most of the other bands completely disappeared from amylose spectrum indicating progressive degradation of its structure. Moreover, the broadening band in the region around 1340 cm^{-1} after heating at 423 K indicated that already at such low temperature disordered carbonaceous material begun to be formed (Beyssac, Goffe, Chopin, & Rouzaud, 2002).

The spectra in Figs. 4a,b and 5a,b showed significant rising (about three times) of the background for thermally treated samples indicating that degradation process led to diminishing of polysaccharides particles (Katumba, Mwakikunga, & Mothibinyane, 2008). Micrographs screening allowed studying the

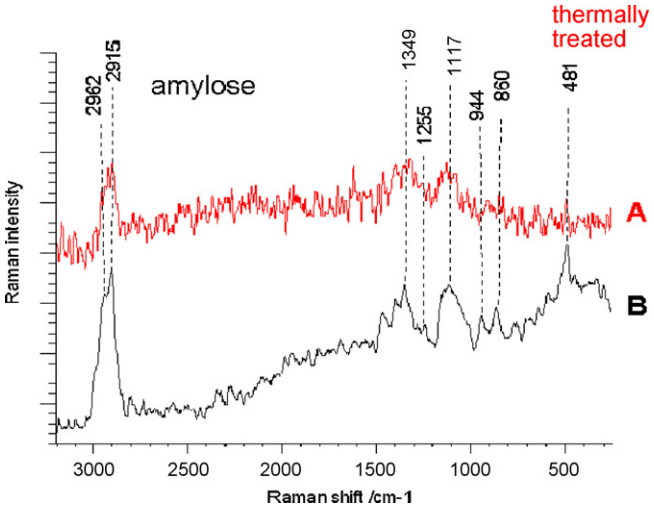

Fig. 3. Raman spectra of amylose, native (B) and thermally treated (A), in the range 3100–200 cm^{-1} (1064 nm excitation).

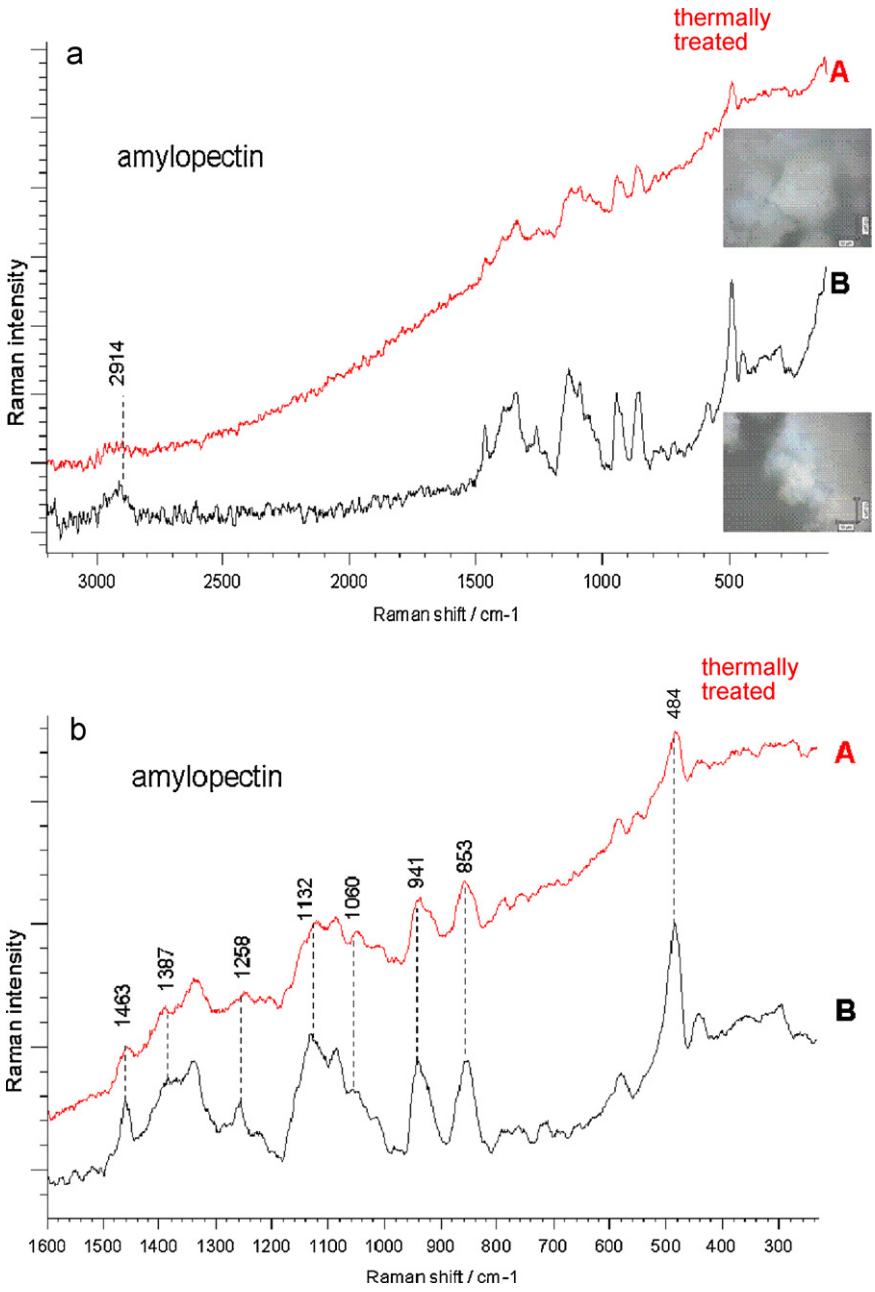

Fig. 4. The Raman spectra of amylopectin, native (B) and thermally treated (A) (785 nm excitation): (a) in the range 3100–200 cm^{-1} and (b) in the range 1600–250 cm^{-1}. Micrographs show samples with 50× magnification, Raman spectrum is taken from the central area of white light image of the size of ca. 1–2 μm.

observed fragmentation of the sample granules into small particles while temperature was rising (Figs. 4a and 5a). The C—C and C—O stretching region (1300–800 cm^{-1}) was very sensitive to the degradation process demonstrating lowering of the intensity of almost all bands. This univocally pointed toward structure modifications, leading to degradation of starch polymers. For example the band at 940 cm^{-1} (ν_s(C—O—C) α-1,4-glycosidic linkage) entirely vanished in amylose, whereas in amylopectin and starch lowered its intensity, what suggested only partial chain splitting. The intensities of the bands at 940 cm^{-1} and 860 cm^{-1} (ν_s(C—O—C) ring mode and C1—H bending α-configuration), in amylopectin decreased in the same way, whereas in starch the glycoside band lowered its intensity in higher degree. Such difference indicated that the easier glycosidic bond cleavage in starch was caused by the presence of amylose.

Thermal treatment affected the band at 1260 cm^{-1}, which was ascribed to a complex mode involving CH_2OH side-chain and the band at 1460 cm^{-1} assigned to the CH_2 twisting mode, both in amylopectin and starch (Figs. 4b and 5b). The observed significant decrease in their intensities might be due to the changes in the hydrogen bond network involving CH_2OH groups. The decrease in intensities observed for bands at 1260, 1084 and 929 cm^{-1} could result from changes occurring in the bending motion of the C—O—H groups.

Raman spectroscopy showed that thermal treatment disturbed all bonds in polysaccharides. The most sensitive was amylose with linear structure, whereas starch, containing high amount of branched amylopectin, with the lowest susceptibility toward temperature, exhibited similar resistance to thermal decomposition, as amylopectin. Observed by Raman spectroscopy violation of

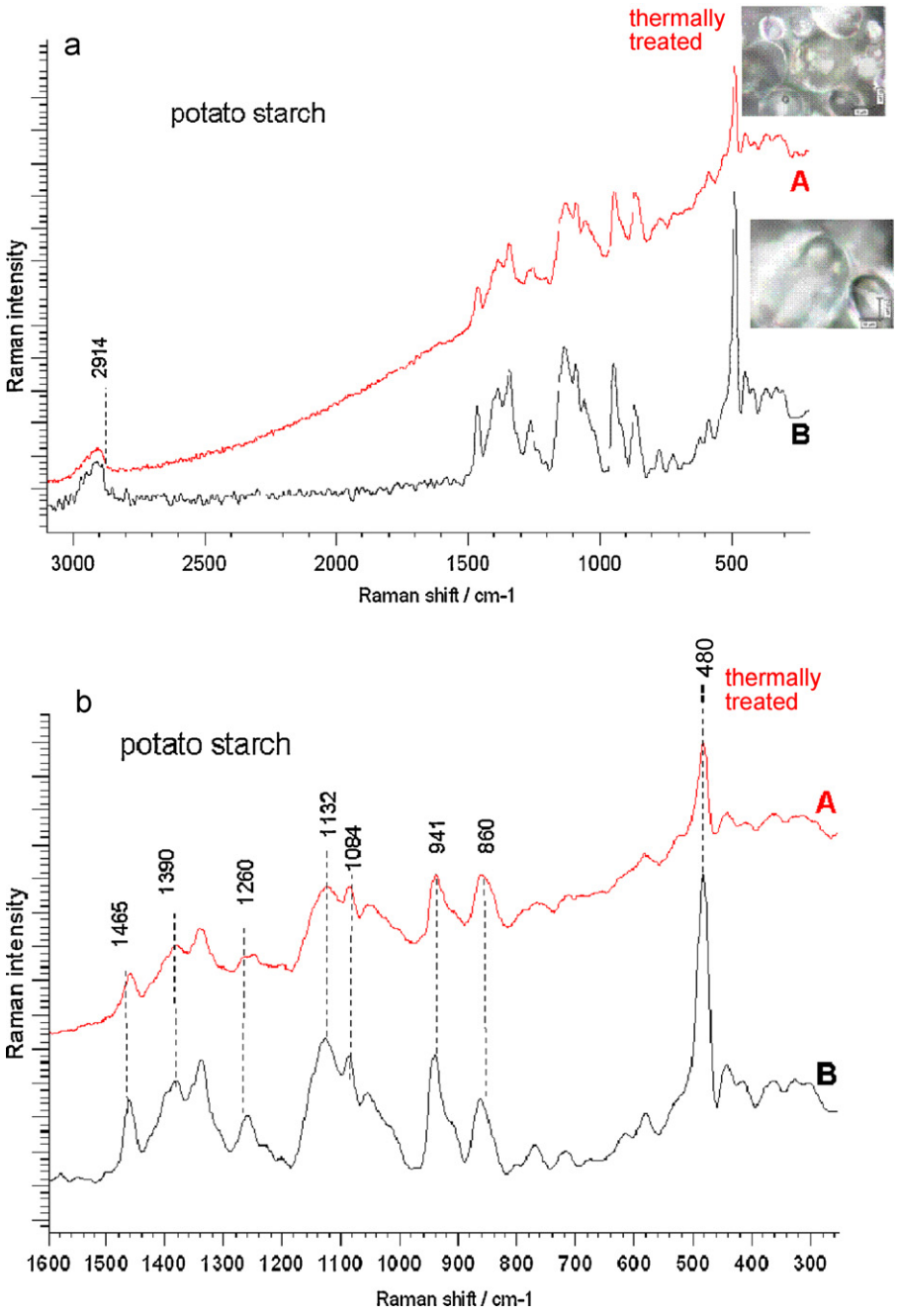

Fig. 5. The Raman spectra of potato starch, native (B) and thermally treated (A) (785 nm excitation): (a) in the range 3100–200 cm^{-1} and (b) in the range 1600–250 cm^{-1}. Micrographs show samples with 50× magnification, Raman spectrum is taken from the central area of white light image of the size of ca. 1–2 μm.

glycosidic bonds, C(1)—H bonds, as well as bonds in group CH_2OH stayed in line with the formation of C(1) and C(6) radicals, which could be considered as an intermediate step of polysaccharides decomposition during thermal treatment.

4. Conclusions

The data obtained from Raman spectroscopy spectra allowed differentiating the susceptibility of potato starch and its fractions to thermal degradation. The most sensitive appeared to be amylose, which molecules subjected to thermal treatment decay already at 423 K. The considerable content of amylopectin in starch accounted for the higher resistance of the latter toward temperature increase. EPR spectroscopy was the more sensitive method to trace the changes occurring in the polysaccharide molecules upon thermal decomposition. It did not only differentiate starch and its fractions by the amounts of thermally generated stable radicals, but also showed their various character and evolution during thermal degradation process. On the basis of EPR results, the structures of created radicals and the sites of bonds cleavage in polysaccharides molecules were proposed.

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