Carbohydrate Polymers 92 (2013) 842–848

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Thermal effects on the structure of cereal starches. EPR and Raman spectroscopy studies

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ARTICLE INFO

Article history:
Received 6 July 2012
Received in revised form
20 September 2012
Accepted 29 September 2012
Available online 8 October 2012

Keywords:
Electron paramagnetic resonance
Raman spectroscopy
Carbohydrate radicals
Corn starch
Wheat starch

ABSTRACT

The mechanism of thermal radical generation has been studied in cereal starches containing different amylose contents. Samples of four corn starches and one of wheat has been investigated using electron paramagnetic resonance and Raman spectroscopies. It has been found, that the content of amylose influences the character of thermally created radical species, but in opposite to potato starch, does not affect their amount. During storage of thermally treated starches the evolution of the EPR spectra has been observed. Raman spectra reveal the smaller changes in cereal starches structure occurring upon high temperature than those found for potato starch.

1. Introduction

Thermal decomposition of starch has been the aim of many studies (Liu, Yu, Liu, Chen, & Li, 2008; Liu et al., 2010; Tomasik, Pałasiński, & Wiejak, 1989). This process engages several reactions and depends on many factors, such as temperature, the presence or absence of air or inert gas and their pressure, moisture content and the amylose/amylopectin ratio (Aggarwal & Dollimore, 1999; Liu, Yu, Liu, Chen, & Li, 2009; Liu et al., 2010). Depending on these factors, different products of thermal degradation are found (Ciesielski & Tomasik, 1996; Tomasik et al., 1989; Zhang, Golding, & Burgar, 2002). At temperatures higher then 400 K, the depolymerisation, followed by the repolymerization occurs with the formation of the new bonds between carbon atoms of glucose units, different than those present in native starch (Zdybel, 2006). Thermal treatment leads also to changes of physicochemical properties, such as gelatinization temperature, viscosity of pastes, gelatinization enthalpy or solubility. In earlier studies we have found that thermal decomposition of starch has been accompanied with formation of radicals (Bidzińska, Dyrek, Fortuna, Łabanowska, & Pietrzyk, 2004; Łabanowska et al., 2008, 2011). Their creation can be considered as one of the stages of starch degradation (Ciesielski & Tomasik, 1996; Tomasik & Jane, 1995; Tomasik et al., 1989). The process of radical generation may be affected by the type of starch structure, the presence and the concentration of functional groups such as —COOH, —CHO, $-PO_4^{3-}$, additional substances (metal ions, sweeteners) (Bidzińska et al., 2004; Blennow et al., 2006; Łabanowska et al., 2008, 2009, 2011; Pietrzyk, Fortuna, & Sowa, 2006; Śmigielska & Lewandowicz, 2007; Śmigielska, Lewandowicz, Goslar, & Hoffmann, 2005; Tolvanen, Sorokin, Mäki-Arvela, Salmi, & Murzin, 2009; Tolvanen et al., 2011).

The structure of starch grains is determined among other things by the content of two starch main fractions amylopectin and amylose. Their different constitutions and molecular weights can cause various course of thermal decomposition. The amylose linear structure facilitates its easier thermal degradation in comparison with highly branched amylopectin (Liu et al., 2009). It is also reflected in the thermal generation of radical species, which are created in higher amount in potato amylose than in amylopectin (Łabanowska, Wesełucha-Birczyńska, Kurdziel, & Sepioło, 2013). Hence, it can be expected that the ratio of both these fractions in particular starches should influence the process of their degradation, as well as the radical creation.

Phosphorus, another natural constituent of starch, affects the starch structure depending on the form of its binding to starch chains. Its content is higher in potato starch than in corn and wheat starches and it exists as phosphate monoesters bound to amylopectin, whereas in cereal starch it forms phospholipids which interact also with amylose (Singh, Singh, Kaur, Sodhi, & Gill, 2003). The presence of phosphate monoesters leads to better water binding capacity, solubility, swelling power and freeze thaw stability (Singh et al., 2003), higher viscosity and transparency (Swinkels,

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0144-8617/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2012.09.087

1985). On the other hand, the formation of complexes between amylose and amylopectin with lipids and phospholipids results in more compact cereal starch structures, what can influence the thermal degradation and radical generation in this starch.

Generally two types of radical species can be distinguished in derived products based on starch: very reactive free radicals, with short life times and the long lived species, which are stable for several days or even months or years. One of the methods especially useful for detection of radicals is electron paramagnetic resonance spectroscopy (EPR), which is not destructive for the sample and exhibits the high sensitivity. It allows investigation of the changes occurring in the radical content and their structures during processes of their formation and vanishing. The short-time radicals can damage the living cells (Ashok & Ali, 1999; Babbs, 1990; Finkel & Holbrook, 2000; Steinberg, 1995) and lead to different diseases. On the other hand, long-lived radicals in polysaccharides can be considered as traps for free electrons, neutralizing in this way the dangerous reactive species (Filek, Kościelniak, Łabanowska, Bednarska, & Bidzińska, 2010; Łabanowska et al., 2012). Therefore, the recognition of the mechanism of stable radicals generation in starch and influence of different factors on this process seems to be essential.

The aim of our work was to investigate the mechanism of thermal generation of long-lived radicals in corn and wheat starches with different amylose content occurring at temperature 483 K and to compare it with the process of radical creation in potato starch.

EPR and Raman spectroscopies were applied in these studies. Electron paramagnetic resonance spectroscopy allowed determining the nature of radicals and their content, whereas Raman spectroscopy gave information on the bond changes occurring in polysaccharide molecules upon high temperature.

2. Materials and methods

2.1. Materials

Corn starch, waxy corn starch Amioca and corn starch Hylon VII were produced by National Starch and Chemical Company, Neustadt, Germany. Wheat starch and another type of corn starch were supplied by Cargill Benelux BV, the Netherlands.

2.2. Content of amylose

Amylose content in the samples was determined spectrophotometrically according to method described by Morrison and Laignelet (1983) with the use of spectrophotometer Genesys 10S (Thermo Scientific, USA).

2.3. Content of total phosphorus

The determination of total phosphorus content was performed according to the Polish norm PN-EN ISO 3946 using spectrophotometer Genesys 10S (Thermo Scientific, USA).

2.4. Thermal treatment

The samples of investigated polysaccharides (about 100 mg) were placed in the quartz EPR tubes and treated in air for 30 min at 423 K, followed by 30 min at 483 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 a X-band Bruker ELEXSYS 500 spectrometer (Karlsruhe, Germany) with 100 kHz field modulation. The spectra were recorded on the day of samples thermal treatment and after one month of their storage in room temperature upon contact with air. The spectra were measured at ambient temperature (293 K) with modulation amplitude of 0.1 mT at microwave power 3.0 mW. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a g-factor standard ($g = 2.0036$). The DPPH with the known number of spins (1.07×10^{15} spins/g) was also used as a quantitative standard of the radical amounts. The maximum error of the quantitative measurements (10 repetitions), calculated for DPPH standard was 0.05×10^{15} spins/g. Quantitative measurements of the samples were repeated three times and averaged. Standard deviation (SE) was estimated for particular samples. The calculated number of spins in each sample was related to the same mass equal 1 g.

EPR parameters such as g factor value and hyperfine splitting constant A were found by a simulation procedure, using the program SIM 32 (Spałek, Pietrzyk, & Sojka, 2005). The accuracy of determination of EPR parameters was ±0.0005 for g values and ±0.1 mT for parameters A.

2.6. Raman spectra

All the Raman spectra were recorded with a Raman microspectrometer model in Via produced by Renishaw (UK) working in confocal mode. Samples were excited with 785 nm laser line of HP NIR diode laser Renishaw (UK). Registrations were repeated five times for each sample. Layer thickness and spot laser size were the same for all samples.

3. Results and discussion

Investigations of thermal radical generation have been carried out for starches containing different amounts of amylose. Four samples of corn starch with different amylose content: National Starch (18.2%), Cargill (21.4%), waxy starch Amioca (1.4%), high amylose starch – Hylon VII (68.7%) have been investigated. Additionally wheat starch with amylose content 19.7% has also been studied.

No EPR signals are observed in all samples before thermal treatment (Fig. 1a – insert). The species giving EPR signals appear in the samples after heating (Fig. 1). The spectra exhibit the similar numbers of radicals, in the range 1.8–3.4 10^{15} per 1 g of sample (Table 1).

The corn and wheat starches give the complex spectra (Fig. 1a–c, g, and h). The simulation procedure reveals that they are composed of four components (centers I–IV). Their characteristics and contribution to particular spectra are given in Table 1. The signal of center I, of the highest intensity, is the doublet. Its width and split depend on the kind of starch and are equal 0.8 mT and 0.5 mT for width and A parameter of signal in wheat starch spectrum, respectively, and 1.0 mT and 0.2 mT for width and A parameter of signal in corn starch spectrum, respectively. The split on two lines is visible only in the simulation spectrum of wheat starch due to the relative values of EPR parameters (Fig. 1, signal I). Signal I may be assigned to the radical formed by abstraction of the hydrogen atom from the carbon atom C(1) of glucose unit (Kuzuya, Yamauchi, & Kondo, 1999, Łabanowska et al., 2008, 2011). The magnetic moment of unpaired electron interacts with nuclear spin of β hydrogen at C(2) giving the signal composed of two lines. The signal originating from center II exhibits four lines of hyperfine structure (HFS) with two different values of splitting indicating the interaction of unpaired electron with two non-equivalent hydrogen atoms (Łabanowska et al., 2011). Such signal was observed previously in potato starch and amylopectin (Łabanowska et al., 2013) and was ascribed to C(6) radical, in which interactions between magnetic moments of an

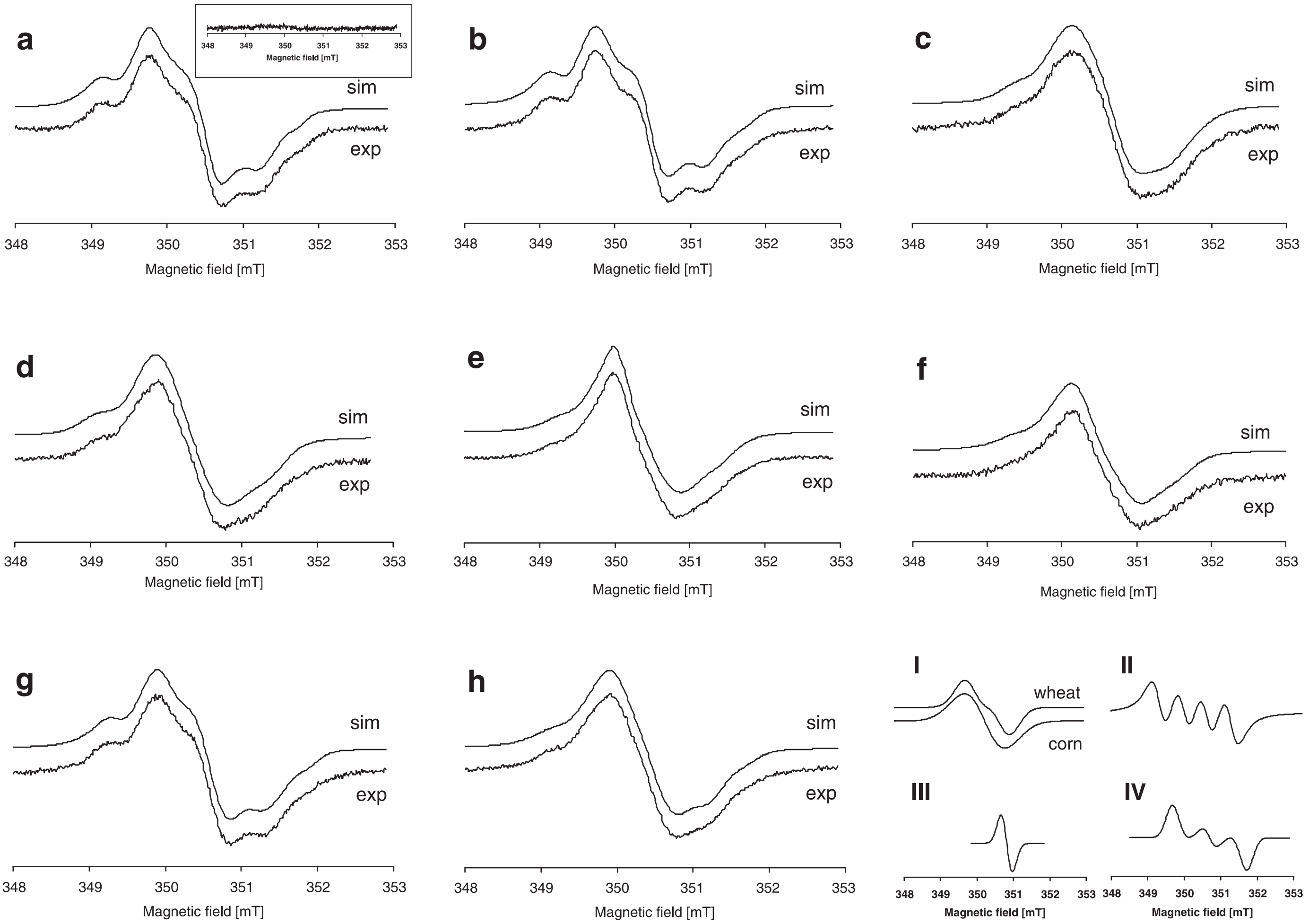

Fig. 1. The experimental and simulated spectra of corn starches and wheat starch registered at 293 K in the range of 5 mT, after thermal treatment of the samples: (a) waxy corn starch, insert – waxy corn starch without thermal treatment (reference line), (b) corn starch (National Starch), (c) high amylose corn starch – Hylon VII, (d) waxy corn starch, registered after 1 month of storage, (e) corn starch (National Starch), registered after 1 month of storage, (f) high amylose corn starch – Hylon VII, registered after 1 month of storage, (g) wheat starch, and (h) corn starch (Cargill), I – IV – particular signals used to the simulation of the spectra.

unpaired electron, localized at this carbon atom, and nuclear spins of α and β hydrogen atoms occurred.

The center III is characterized by the single line with lower g factor value than that of signals I and II. It points that the unpaired electron is not influenced by oxygen atom and similarly as it has been found in our previous paper (Łabanowska et al., 2013) can be attributed to electron delocalized on the system of conjugated carbon bonds. Such system can be formed as a result of gradual dehydration of polysaccharide molecules. The detailed studies on the formation of particular signals in potato starch with different content of amylose, as well as in pure amylose, at various heating temperature (423–503 K) (Łabanowska et al., 2013) allow finding that during prolonged heating at raising temperature, when process of dehydration is intensified, signal III increases its intensity at the cost of signal I. Such phenomenon can be explained assuming that during dehydration the β hydrogen atom, responsible for HFS structure of signal I, forms water molecule with OH group situated at C(3) and is removed from glucose unit. In the consequence the double bond between C(2) and C(3) is created and simultaneously HFS structure of signal I disappears. In the case of potato starch further increase of temperature (up to 503 K) leads to the strong decrease of the contribution of signal I to the integral intensity of the spectrum and is accompanied with the increase of the contribution of signal III. The g factor of signal III in the spectra of both potato and cereal starches achieves similar values, lower than those of signal I confirming the weaker influence of oxygen on an unpaired electron suggesting structure of glucose ring depleted in oxygen, with double carbon—carbon bonds.

The good fitting of theoretical line to experimental one of corn and wheat starch spectra requires the assumption of the presence of additional three doublets with various values of splitting (signal IV). The low g factor points that this radical is probably created on a carbon atom not adjacent to any oxygen atoms. Such radical may be generated by abstraction of OH group from C(6), and coupling between magnetic moments of an unpaired electron and nuclear spins of two α hydrogen atoms and β hydrogen at C(5) leads to signal with hyperfine structure. The formation of radical at C(2) or C(3) also by abstraction of OH group may result in the similar signals.

The radicals generated upon thermal treatment of all starches exhibit the similar EPR characteristics (Table 1), however, the contributions of particular signals to integral intensities of the spectra are different. Contribution of signal I to spectra of starch recorded directly after thermal treatment varies in the range 62–75% and appears to be rather independent of amylose content in the samples (Table 1). It reaches 75% in Hylon and 70% in waxy corn. It can point, that this center is created both in amylose and amylopectin structures, similarly as it is observed for potato starch (Łabanowska et al., 2013). The contribution of signal II is connected with the amount of amylopectin fraction. Participation of this signal is twice lower in

Table 1
EPR parameters of the signals found from simulation procedure in the samples of corn starches and wheat starch after thermal treatment. g – value of g-factor, A_1–A_3 – values of hyperfine splitting, ±SE – standard error.

	Number of spins/1 g (±SE)	g	A_1 [mT]	A_2 [mT]	A_3 [mT]	Contribution [%]	Signal	Attribution
Corn starch (National Starch)	$2.9 \times 10^{15} \pm 0.08 \times 10^{15}$	2.0054	0.2			62	I	C(1)
		2.0064	0.6	1.2		20	II	C(6)
		2.0046				9	III	Delocalised[a]
		2.0034	0.5	1.0	0.3	9	IV	C(6)
Corn starch (Cargill)	$2.2 \times 10^{15} \pm 0.09 \times 10^{15}$	2.0054	0.3			71	I	C(1)
		2.0065	0.7	1.1		14	II	C(6)
		2.0038				7.5	III	Delocalised[a]
		2.0042	0.5	1.1	0.3	7.5	IV	C(6)
Corn starch (National Starch) after 1 month storage	$2.3 \times 10^{15} \pm 0.06 \times 10^{15}$	2.0057				86	I	C(1)
		2.0064	1.0	1.0		14	II	C(6)
Waxy corn starch	$3.4 \times 10^{15} \pm 0.07 \times 10^{15}$	2.0054	0.3			70	I	C(1)
		2.0064	0.6	1.2		18	II	C(6)
		2.0044				6	III	Delocalised[a]
		2.0032	0.5	1.0	0.2	6	IV	C(6)
Waxy corn starch after 1 month storage	$2.7 \cdot 10^{15} \pm 0.10 \cdot 10^{15}$	2.0053	0.5			86	I	C(1)
		2.0062	0.8	1.1		14	II	C(6)
Corn starch Hylon	$2.1 \times 10^{15} \pm 0.14 \times 10^{15}$	2.0052	0.4			75	I	C(1)
		2.0065	0.8	1.0		9	II	C(6)
		2.0045				11	III	Delocalised[a]
		2.0035	0.4	0.8	0.2	5	IV	C(6)
Corn starch Hylon after 1 month storage	$1.8 \times 10^{15} \pm 0.10 \times 10^{15}$	2.0048	0.5			92	I	C(1)
		2.0058	0.8	1.0		8	II	C(6)
Wheat starch	$2.5 \times 10^{15} \pm 0.08 \times 10^{15}$	2.0056	0.5			64	I	C(1)
		2.0067	0.6	1.2		18	II	C(6)
		2.0046				9	III	Delocalised[a]
		2.0030	0.4	1.0	0.3	9	IV	C(6)

[a] Delocalised on conjugated double bonds.

Hylon spectrum than in corn, wheat and waxy corn starches spectra (Table 1). It confirms, that like in potato starch (Łabanowska et al., 2013), signal II is created mainly in amylopectin fraction. Comparing the radicals amounts in pure fractions of potato starch we have found, that significantly higher number of radicals are created in amylose than in amylopectin. In corn starches, we do not observe important differences in radical content between Hylon and waxy corn, and even, at the same conditions, the higher number of radicals is formed in waxy corn, rich in amylopectin (Table 1).

Remaining signals, III and IV, exhibit smaller contributions to starch spectra. It seems that their intensities are only slightly dependent on the content of amylose. In potato starch signal III contributes in significant degree to the amylose spectrum and is the one which differentiates mostly the particular fractions: amylose and amylopectin. For samples obtained at the same conditions (483 K), it exhibits the highest contribution to the spectrum of amylose fraction, whereas it is absent in the spectrum of amylopectin (Łabanowska et al., 2013). The lack of such dependence in corn starches indicates that not only the ratio of amylose to amylopectin, but also others factors can influence the process of radical generation. One of them seems to be the content of phosphorus, higher in potato starch (0.085 g/100 g) than in corn (0.020 g/100 g). The repulsion between phosphate groups on adjacent chains of amylopectin causes weakening or breaking of bonds in amylopectine fraction (Singh et al., 2003). This effect probably facilitates the radical generation in potato starch. On the other hand, the presence of phosphorus in phospholipid form in cereal starches inhibits radical creation. Phospholipids and lipids present in cereal starches form complexes with amylose fraction and long chains of amylopectin, which cause stronger interactions between starch chains within the amorphous (amylose) and crystalline (amylopectin) domains (Singh et al., 2003). Such interaction can hinder the formation of carbohydrate radicals. The other factor which determines the difference between courses of radical generation processes appears to be the starch structure. The structure of cereal starch (type A), more packed than structure of potato starch (type B) (Kuakpetoon & Wang, 2006), hampers the decomposition of starch and inhibits the radical creation. Although radical generation processes occur in various way in particular kinds of starch, the mechanism of their formation, engaging the same carbon atoms, is similar. Our studies show that cereal starches appear to be more complicated systems than potato ones. The presence of lipids and phospholipids interacting with amylose, obstructs its ability to degradation causing that Hylon, containing high amount of amylose fraction, is in its thermal characteristics more similar to amylopectin than to potato amylose. This observation can lead to the conclusion that contrary to potato starch, where the content of amylose controls the process of radical formation, in cereal starches amylopectin fraction is more engaged in the thermal generation of radicals. It is reflected in the contributions of signals II and III to the spectra of cereal and potato starches, created at the same conditions. The contribution of signal II to the spectrum of cereal starch related to amylopectin fraction is twofold higher, than in case of potato starch, whereas the contribution of signal III created mainly in amylose is twofold higher to the spectrum of potato starch than to cereal one (Łabanowska et al., 2013).

The registration of the spectra of thermally treated corn starch, waxy corn and Hylon after one month of storage at room temperature with air access, reveals that after this time the radical composition of samples changes (Fig. 1d–f, Table 1). In all spectra the centers III and IV vanish. The disappearance of both centers is probably caused by water absorption, which leads to formation of the bond C(6)—OH and saturation of the double bonds, formed in the glucose units during thermal treatment. On the other hand, signal I increases its contribution to the spectra, which probably is a result of its reproducing from signal III. Participation of signal II

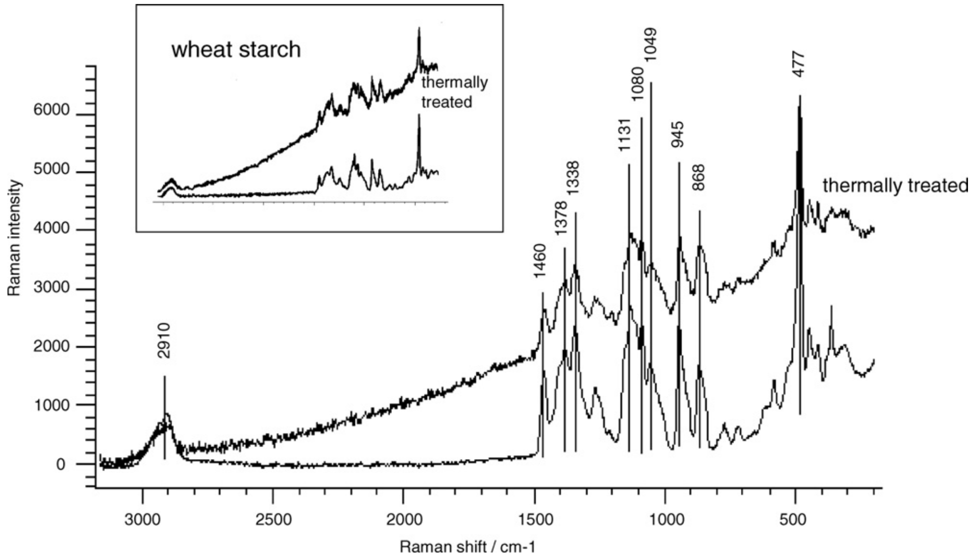

Fig. 2. Raman spectra of corn starch, native and thermally treated, in the range 3100–200 cm^{-1}. In insert – spectra of wheat starch, native and thermally treated.

undergoes the least changes during sample storage indicating that centers created in amylopectin structure are more stable. Moreover, equalization of A parameters of this signal occurs, which can suggest the shortening of C(5)—C(6) bond, elongated during thermal treatment.

The Raman spectra of the starch samples taken before and after thermal treatment are presented in Figs. 2–4. The spectra of unheated starches are very much alike. After thermal treatment, the most characteristic feature of all the spectra is the significant rising of the background. It indicates that degradation process taking place in high temperature diminishes the length of polysaccharide particles (Katumba, Mwakikunga, & Mothibinyane, 2008). The region at c.a. 3000 cm^{-1}, which was O—H and C—H stretching region, is rather poorly resolved. In the region below 1700 cm^{-1} several bands are visible. The observed modes are similar to those characteristic for glucose molecule, the unit building starch chains (Cael, Koenig, & Blackwell, 1973). In all the spectra the highest intensity shows a band at 478 cm^{-1}. This band is attributed to skeletal mode involving (C—O—C) ring mode and δ(C—C—O). Other bands, characteristic for polysaccharides, which can be found in the spectra are gathered in Table 2 and shown in Figs. 2–4 (Cael et al., 1973; Kizil, Irudayaraj, & Seetharaman, 2002; Passauer, Bender, & Fischer, 2010; Sekkal, Dincq, Legrand, & Huvenne, 1995; Soderholm, Roos, Meinander, & Hotokka, 1999). Raman spectroscopy shows that thermal treatment affects all the bands observed in the spectra. Their intensities decrease, showing that all the bonds existing in investigated polysaccharide molecules are being destroyed at high temperature.

The detailed analysis of Raman spectra reveals, that among bands, which diminish mostly after thermal treatment, are the bands 1150 cm^{-1} and 944 cm^{-1} assigned to ν_a and ν_s of (C—O—C) α-1,4-glycosidic linkage. It points that during thermal treatment the cleavage of this bonds and decomposition of polysaccharide chains takes place. Other bands which are more affected by temperature are the bands 477 cm^{-1} and 1130 cm^{-1}. The strong decrease of the band at 477 cm^{-1} indicates disarrangement of the starch structure at the level of glucose ring. Significant decrease of 1130 cm^{-1} band attributed to ν(C—OH) bending, ν(C—O), δ(C—OH) can be explained by dehydration of the starches. It is worthwhile to notice that the band at 1341 cm^{-1} has changed more during heating in waxy corn than in starches with lower amylopectin

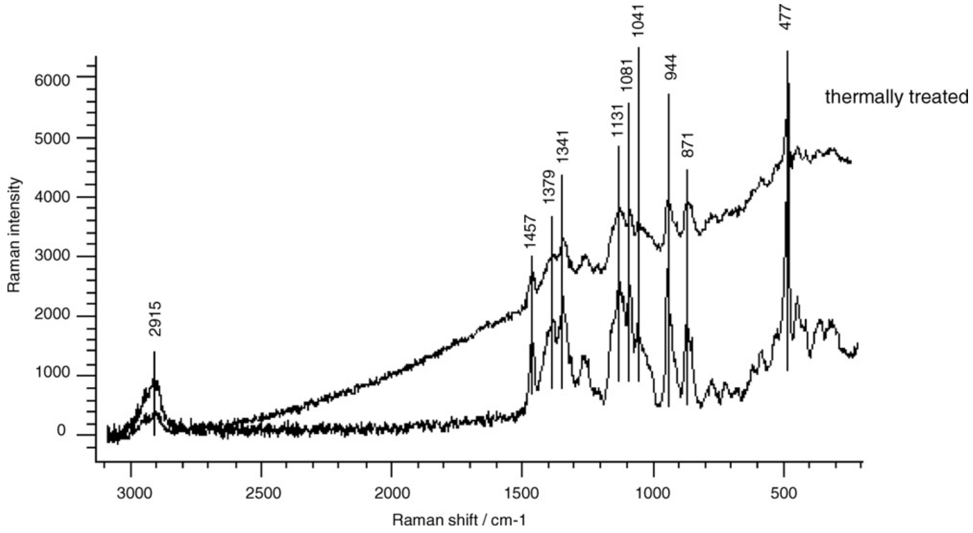

Fig. 3. The Raman spectra of waxy corn starch, native and thermally treated, in the range 3100–200 cm^{-1}.

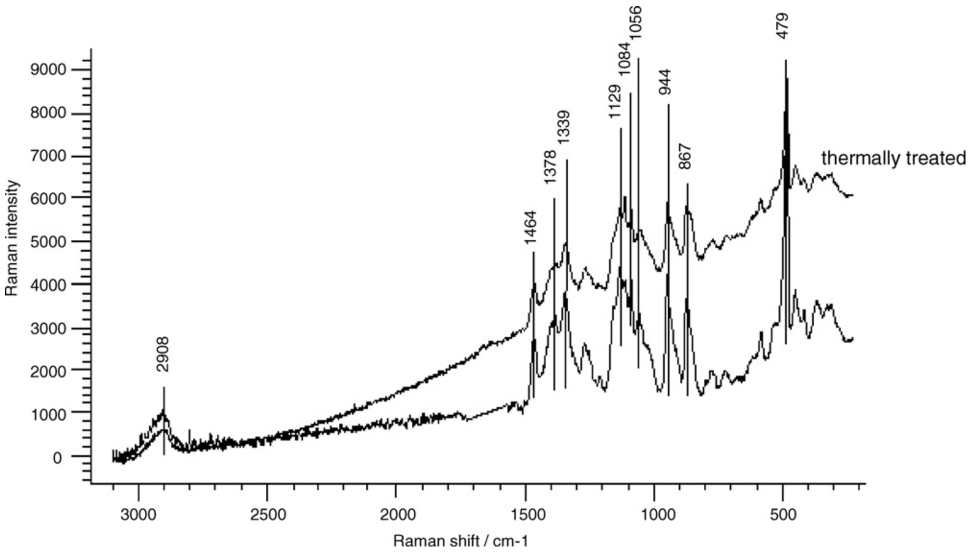

Fig. 4. The Raman spectra of high amylose corn starch Hylon VII, native and thermally treated, in the range 3100–200 cm^{-1}.

Table 2
Positions [cm^{-1}] and assignments of Raman bands of corn starches and wheat starch before and after thermal treatment (Cael, Koenig, & Blackwell, 1973 –a; Colthup, Wiberley, & Daly, 1964 –b; Passauer, Bender, & Fischer, 2010 –c; Sekkal, Dincq, Legrand, & Huvenne, 1995 –d; Soderholm, Roos, Meinander, & Hotokka, 1999 –e).

Hylon native	Hylon treated at 483 K	Waxy corn starch native	Waxy corn starch treated at 483 K	Corn starch native	Corn starch treated at 483 K	Wheat starch native	Wheat starch treated at 483 K	Assignment
2908	2908	2915	2913	2910	2908	2910	2911	$\nu_{as}(CH_2)$; (b)
1464	1463	1457	1455	1460	1459	1462	1460	$\delta(CH_2)$ twisting, CH bending; (c, d)
1378	1377	1379	1378	1378	1378	1382	1379	δ(C—H), CH bending, (a), CH scissoring
1339	1340	1341	1342	1338	1338	1340	1340	$\delta(CH_2)$, C—OH bending; (c, d)
1260	1260	1261	1260	1262	1262	1261	1259	$\delta(CH_2)$, CH_2OH (side chain) related mode; (a)
1210			1213	1211	1205		1210	δ(C—H); (c), δ(C—O—H); (e)
1150 sh	1150 sh	1150 sh	1150 sh	1150 sh	1150 sh	1150 sh	1150 sh	ν_a(C–O–C) α-1,4-glycosidic linkage;(b)
1129	1129	1131	1130	1131	1130	1131	1132	ν(C—OH) bending, ν(C—O), δ(C—OH); (b)
1084	1084	1081	1081	1080	1079	1084	1084	ν(C—O—C) ring mode, C—OH bending; (c, d)
1056	1055	1041	1041	1049	1048	1049	1048	δ(C—OH), ν(C—OH); (b)
944	944	944	944	945	944	943	942	ν_s(C—O—C) α-1,4-glycosidic linkage;(a)
867	867	871	870	868	868	869	867	ν_s(C–O–C) ring mode, C1—H bending α-configuration; (b)
765	762	760	762	770		762	760	ν(C—O); (a)
580	582	580	580	582	580	580	580	Skeletal modes; (b)
479	480	477	477	477	478	478	478	skeletal mode involving (C—O—C) ring mode, δ(C—C—O); (b)
450	450	445	443	445	445	450	450	δ(C—C—C); (e)
418	418	412	412	414	418	408	408	δ(C—C—O); (e)

content. It can be connected with the radical formation at C(6), which occurs with higher efficiency in amylopectin fraction.

In this study we observe, that the spectra of all corn starches change in the similar way after thermal treatment. In our previous work (Łabanowska et al., 2013) we found quite different behavior of amylose of potato origin. In the spectrum of heated potato amylose sample most of the bands completely disappear, indicating advanced degradation of amylose structure. The presence of amylopectin, as well as the lipid and phospholipid complexes with amylose in Hylon sample results in its higher resistance to thermal decomposition. About threefold higher amylose amount in Hylon compared to corn starch (National Starch) do not affect significantly its behavior during thermal treatment.

4. Conclusions

Raman and EPR spectra show that the amylose content influences the thermal degradation and the radical generation in cereal starches only slightly, contrary to the process observed during thermal decomposition of potato starch. Such difference between both kinds of starch results from various structure of starches, their composition and character of phosphorus bonding. In cereal starch the presence of complexes of amylose and amylopectin with lipids/phospholipids hinders the radical formation. Hence, the process of their generation occurs with limited amylose participation. The course of radical creation is reflected in the contribution of particular signals to the EPR spectra. However the radical generation proceeds in various ways in cereal and potato starch, the nature and EPR parameters of created radical species are similar in all kinds of starches indicating that mechanism of their formation involves the same carbon atoms of glucose units. The radicals at C(1) are generated with the highest efficiency in both, amylose and amylopectin fraction, whereas those at C(6) are formed in amylopectin fraction with greater yield in cereal starches. The main difference between the mechanism of radical creation in starches of various origin consists in the rate of transformation of the radical located at C(1) to the radical stabilized by conjugative double bonds. It occurs mainly in amylose, therefore in cereal starches such stabilization is inhibited due to amylose complexation by phospholipids/lipids.

Acknowledgment

The authors wish to thank Cargill Benelux BV, the Netherlands for sending free samples of their products.

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