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Effect of high hydrostatic pressure on the formation of radicals in maize starches with different amylose content

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ABSTRACT

Native and high pressure-treated (water suspensions, 650 MPa) waxy maize starch, containing mainly amylopectin, and Hylon VII, rich in amylose, were studied for their ability to generate free radicals upon thermal treatment at 180–230 °C. The electron paramagnetic resonance (EPR) spectroscopy was used to characterize the nature, number and stability of radicals. Various stable and short living (stabilized by *N-tert*-butyl-α-phenylnitrone (PBN) spin trap) radical species were formed. It was found, that at given conditions the waxy maize starch reveals higher ability to generate radicals, than Hylon VII. The presence of water and high pressure pretreatment of starches, both resulted in the reduction of the amount of thermally generated radicals. The decrease in crystallinity of waxy maize starch and of Hylon VII, occurring upon high pressure treatment, leads to the increase of the relative amount of fast rotating component in the EPR spectrum of both types of starches.

1. Introduction

It is generally believed, that some methods of starch modification, e.g., irradiation, lead to free radicals formation (Bertolini, Mestres, Colonna, & Raffi, 2001). It is also well known, that the thermal treatment of starch causes degradation of its structure and generation of radicals (Ciesielski, Achremowicz, Tomasik, Baczkowicz, & Korus, 1997; Ciesielski & Tomasik, 1996). Treatment of starch with high hydrostatic pressure may evoke, already at room temperature, significant changes in the granule structure, as shown by Rubens and Heremans (2000) and Stolt, Oinonen, and Autio (2001). X-ray studies demonstrated, that the high pressure treatment of starch granules in excess of water may lead to the changes in the degree of crystallinity (Błaszczak, Fornal, & Valverde, 2005a; Błaszczak, Fornal, Valverde, & Garrido, 2005b) and/or to the change in relative content of starch crystalline polymorphs (A and B types) (Katopo, Song, & Jane, 2002). These effects depend strongly on the starch composition, i.e., on the content of amylose and amylopectin. Thus, e.g., starch rich in amylose (Hylon VII) subjected to high hydrostatic pressure (Błaszczak et al., 2005a, b) demonstrated only partial decrease in the degree of crystallinity, whereas the waxy maize starch, containing mostly amylopectin, after treatment at similar conditions revealed a fully amorphous character. The changes in the degree of the crystallinity occurring upon high pressure are often accompanied by modifications of some morphological, thermal and osmotic properties of the starch (Błaszczak, Fornal, et al., 2007; Błaszczak et al., 2005a, b; Błaszczak, Misharina, Yuryev, & Fornal, 2007).

Taking into account the above mentioned effects it could be expected that pressurized starches may exhibit ability to free radical formation and that this feature will depend on the amylose to amylopectin ratio. The presence of free radicals is not irrelevant for the quality of food because it may cause various diseases (Babbs, 1990; Steinberg, 1995) and accelerate negative cellular changes associated with aging (Ashok & Ali, 1999). Especially dangerous might be short living, very reactive radicals.

The present studies were addressed to the effect of the high hydrostatic pressure and amylopectin/amylose content on the ability of free radicals formation by waxy maize and Hylon VII starch. It is worthy mentioning that the radicals were generated in the studied samples in the temperature range 180–230 °C, commonly used for food preparation. The electron paramagnetic resonance (EPR) spectroscopy was applied to determine the nature of radicals, their number and stability. A spin trap, *N-tert*-butyl-α-phenylnitrone (PBN), was used for detection and stabilization of especially reactive, short living radicals and for monitoring of the starch lattice dynamics. The aim of the work was to get data useful for control-

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ling the process of radical formation, which may influence quality of food products containing starch.

2. Materials and methods

2.1. Materials

The experimental material was Hylon VII (containing 68% of amylose) refined from high amylose maize produced by National Starch and Chemical, Food Starch, Poland and waxy maize starch (amylopectin with trace amounts of amylose) purchased from Sigma (S-9679).

2.2. Methods

2.2.1. Pressure treatment

The pressure treatment of starches was performed in the excess of water, i.e., using 30% (w/w) starch–water suspensions closed in teflon tubes (10 mL), precisely mixed, deaerated, sealed and pressure-treated (Błaszczak, et al., 2005a). The treatment was performed in a high pressure device (high pressure press type LV30/16, produced by The Centre of High Pressure Analysis, Polish Academy of Sciences, Warsaw, Poland).

The teflon tubes were put into a high pressure chamber (with the capacity of approximately 25 mL), filled with pressure-transmitting medium, which also minimized adiabatic heating. The samples were pressure-treated at 650 MPa for 9 min. The time for reaching the working pressure was 120 s. The temperature inside the pressure chamber averaged 20 ± 2 °C. Pressurized starch pastes and gels were dried at room temperature in a desiccator first under the reduced pressure and finally over P_2O_5. The dry samples were pulverized with an agate mortar. The non-pressurized waxy maize and Hylon VII samples, used as references for EPR measurements, were treated in a similar way as the pressurized ones, i.e., transformed into suspensions and dried in a desiccator under the reduced pressure and P_2O_5.

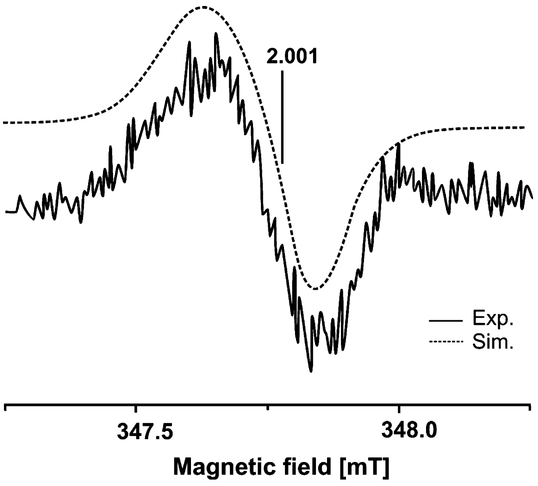

Fig. 1. EPR signal (registered at 20 °C) of radicals generated in native Hylon VII by high pressure treatment at room temperature.

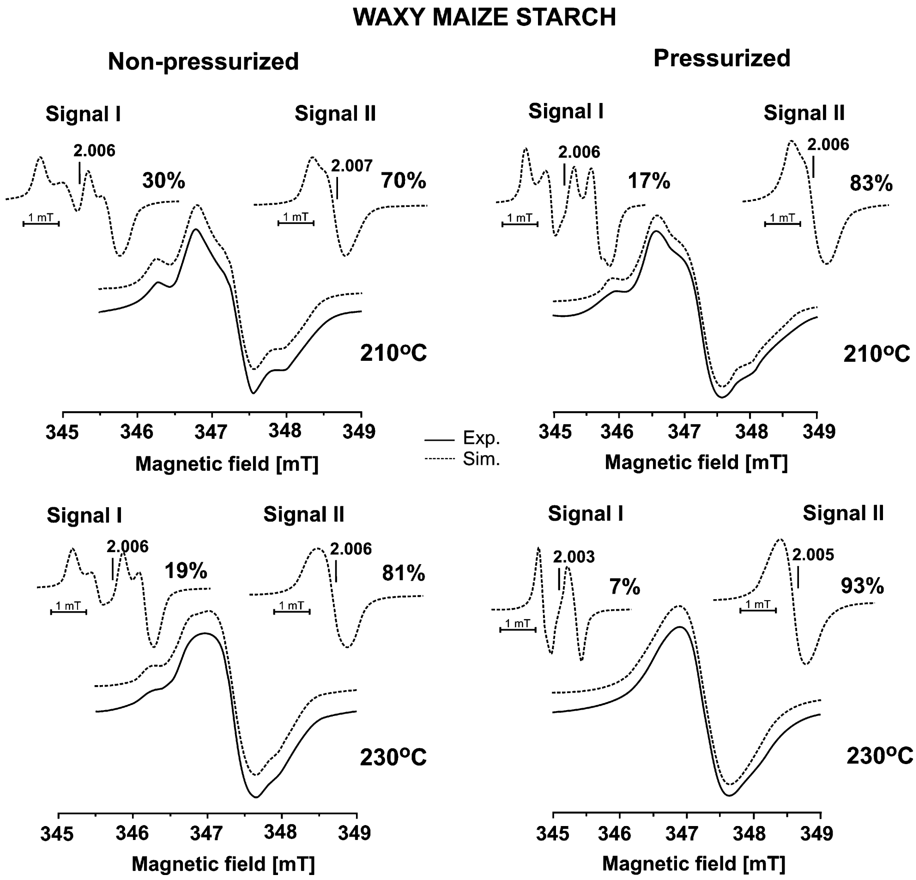

Fig. 2. Influence of the temperature of heating and high pressure treatment on the shape of EPR signals (registered at 20 °C) of radicals generated thermally in waxy maize starch.

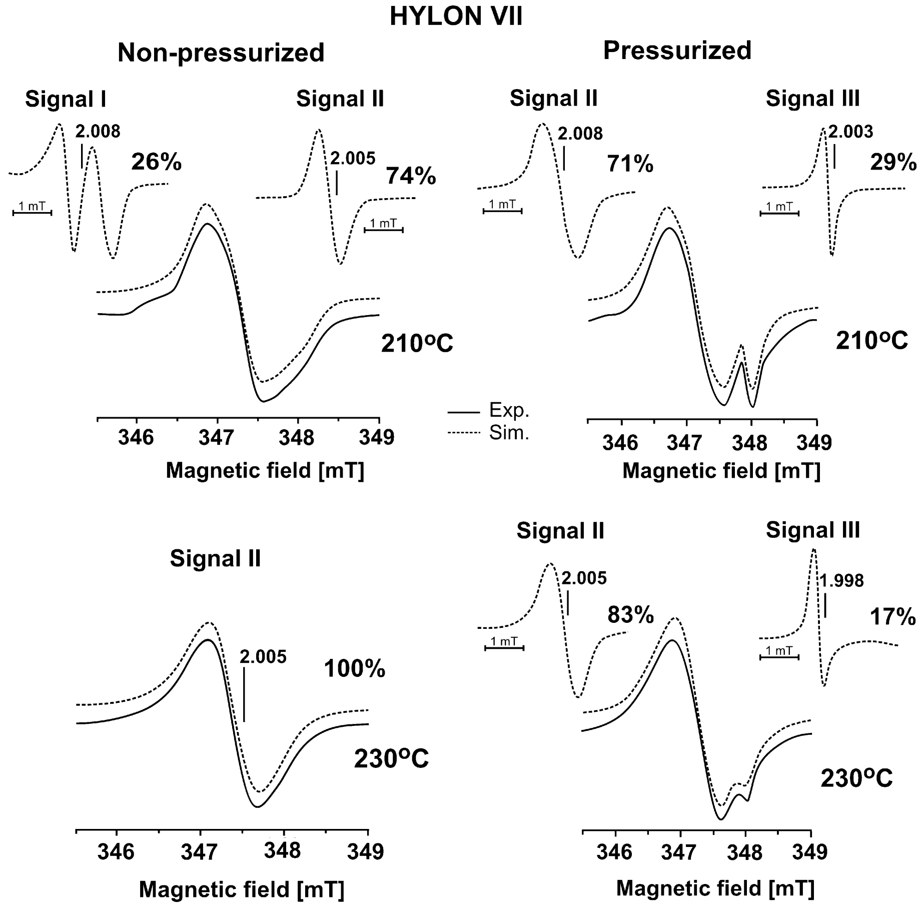

Fig. 3. Influence of the temperature of heating and high pressure treatment on the shape of EPR signals (registered at 20 °C) of radicals generated thermally in Hylon VII.

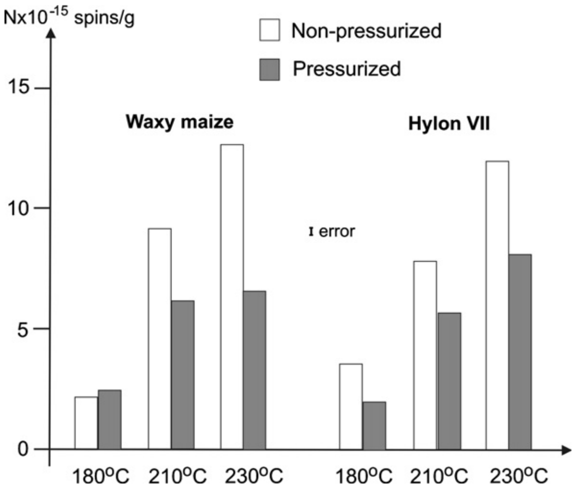

Fig. 4. Influence of the temperature of heating and high pressure treatment on the number of radicals generated thermally in waxy maize starch and in Hylon VII.

2.2.2. Thermal treatment

Starch samples of about 30 mg were placed in EPR quartz tubes (inner diameter = 3 mm) and heated in an oven for 30 min at a given temperature (in the range 180–230 °C). During heating the tubes were open, i.e., the samples were in contact with air. After treatment the tubes were closed with a paraffin membrane.

2.2.3. Determination of the content of water

Content of water in the non-pressurized and pressurized starches was determined as the weight loss of the samples heated at 110 °C until they exhibited constant weight.

2.2.4. EPR technique

EPR measurements were performed at room temperature with a Bruker ELEXSYS 500 spectrometer (Karlsruhe, Germany) operating in X-band (9.2 GHz) at modulation frequency 100 kHz, modulation amplitude 0.3 mT and microwave power 3 mW. The number of spins was determined by comparison of the integral signal intensity of the investigated samples with that of the standard with the known amount of paramagnetic centers. All necessary precautions, discussed in publications (Dyrek, Madej, Mazur, & Rokosz, 1990; Dyrek, Rokosz, & Madej, 1994) were followed, in order to assure good precision of the quantitative EPR measurements. $VOSO_4 \cdot 5H_2O$ diluted with diamagnetic K_2SO_4, containing 5×10^{19} spins/g, was used as a primary standard. Generation of radicals was investigated on native and pressurized samples before and after heat treatment.

N-tert-butyl-α-phenylnitrone (PBN) was used as a spin trap, to detect short living paramagnetic species. Ethyl alcohol solution of PBN was added to the water suspensions of the starch (50 μL of 1 M PBN solution per 1 mL of the 30% starch suspension) before appropriate treatment and then EPR measurements of starch/PBN blends were performed. The samples containing spin trap were heated at relatively low temperature (180 °C), because of the low thermal stability of PBN whereas the samples without PBN were heated in the temperature range 180–230 °C.

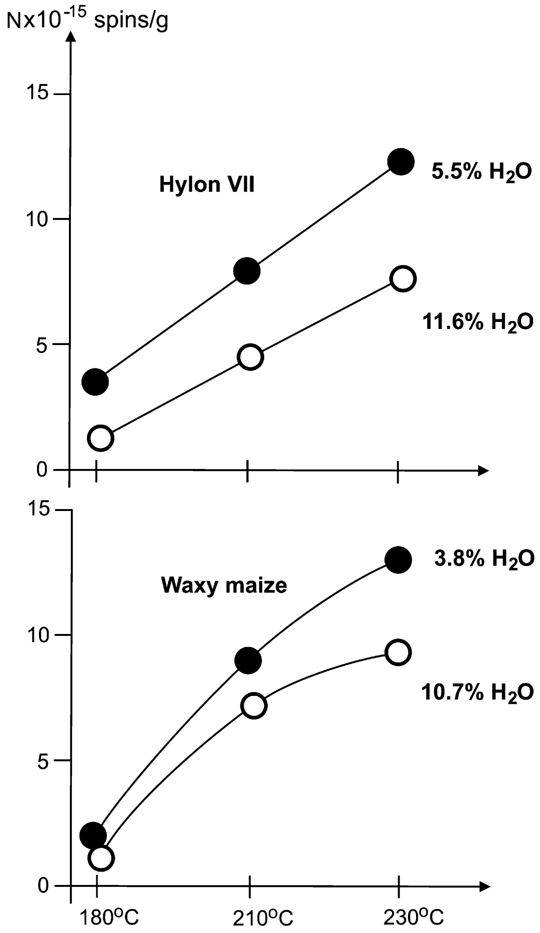

Fig. 5. Influence of the content of water in waxy maize and Hylon VII on the number of thermally generated radicals.

EPR parameters of the radicals were determined by a simulation procedure using the program SIM 32 (Spałek, Pietrzyk, & Sojka, 2005). The accuracy of determination of g values was ±0.001 and that of A values was ±0.1 mT.

3. Results and discussion

3.1. EPR spectra before heat treatment

The native waxy maize and Hylon VII starches did not show any EPR signal. After high pressure treatment at room temperature only Hylon VII samples exhibited a small EPR signal with $g = 2.001$ (Fig. 1) and intensity equal to about 10^{13} spins/g, indicating formation of paramagnetic defects. The number of spins related with this signal was of two orders of magnitude smaller than that of thermally generated radicals, as will be shown hereafter.

3.2. EPR spectra after heat treatment

The samples of waxy maize and Hylon VII starch, non-treated and treated with the high pressure, revealed, after heating in the temperature range 180–230 °C, the presence of EPR signals indicating formation of thermally generated radicals. The EPR parameters of the signals and their intensities differed depending on temperature, type of the starch and applied treatment (Figs. 2–5, Table 1).

The radicals generated in native waxy maize starch give EPR spectra at room temperature with two components (signal I and II), similar to those observed for native potato starch (Dyrek et al., 2007). The radicals with signal I are generated thermally by abstraction of hydrogen ($H^{\cdot}$) from C_1 atom of the glucose unit (Fig. 6). This signal with $g_{av} = 2.006$ exhibits hyperfine structure (HFS) with $A_{av} = 1.1 - 1.2$ mT (Fig. 2, Table 1), indicating interaction of unpaired electron localized on C_1 carbon atom with nuclear spin of β hydrogen (localized at C_2). Signal II, without HFS, with g_{av} value equal to 2.006–2.007, represents carbon radical not interacting with neighboring hydrogen. The formation of this radical requires simultaneous abstraction of hydrogen and dehydration (Fig. 6). The relative content of the radical with signal I decreased from 30% at 210 °C to 19% at 230 °C (Table 1). Similar effect, indicating more efficient abstraction of β hydrogens from the starch structure at elevated temperatures, was observed for native potato starch (Dyrek et al., 2007).

The type of the EPR signals in pressurized waxy maize starch heated at 210 and 230 °C is the same as in the native samples, however, the content of radicals with signal I is smaller, amounting 17% and 7%, respectively. This fact indicates that the high pressure favors abstraction of β hydrogen from the glucose unit.

In non-pressurized Hylon VII heated at 210 °C similar radicals, but with smaller content of radical I, were observed, whereas after heating at 230 °C only signal of radical II, without HFS, was found (Fig. 3 and Table 1). Most probably, abstraction of β hydrogen and dehydration from amylose (i.e., from Hylon VII) is easier than from amylopectin (i.e., from waxy maize starch), which causes depletion of the vicinity of unpaired electron from β hydrogens in amylose-rich Hylon VII. In pressurized Hylon VII radicals of type II were formed at both temperatures (71–83%) and, additionally, a radical with slightly anisotropic EPR signal and g factor close to 2.0 (signal III), similar to that generated in Hylon VII by high pressure at room temperature (Fig. 1).

The total intensity of EPR signals of radicals in both types of the starch increases with increasing temperature (Fig. 4) and the signals became more symmetric (Table 1, Figs. 2 and 3). Decreasing anisotropy of radicals generated at higher temperature is probably due to the better removing of the traces of water which provides more space for rotation of paramagnetic species. The shape and intensity of the signals do not change significantly after two months of storing in air at room temperature, indicating their relatively good stability. The number of radicals generated at 210–230 °C is higher for native waxy maize starch than for native Hylon VII (Fig. 4), which may be caused by different content of water, amounting 3.8% for waxy maize and 5.5% for Hylon VII. It is known from the literature, that the presence of water accelerates disappearance of radicals generated in starch by irradiation (Bertolini et al. 2001; Lee & Bhardwaj 1964) or by heating at elevated temperatures (Ciesielski & Tomasik 1996). To check, if the observed difference in the number of radicals for two types of the starch is caused by water the experiments of radical generation by heat treatment were performed with waxy maize and Hylon VII samples containing various amount of moisture. The results presented in Fig. 5 clearly indicate, that in the samples containing less water higher number of radicals were formed at a given temperature, which confirms our supposition of the important role of water in determination the number of radicals generated thermally in starch.

Significant influence on the content of radicals has also pretreatment of the starch samples with high hydrostatic pressure – the number of radicals drastically diminish after pressurization (Fig. 4). It may be supposed, that the effective reduction of the number of radicals is due to the water molecules squeezed into the starch granules upon treatment of the starch–water suspensions with high hydrostatic pressure. In fact, Katopo et al. (2002) observed the increase in the relative content of B-type crystalline polymorph, containing more water molecules per unit cell, occurring upon hydrostatic pressure (Katopo et al. 2002).

Table 1
EPR parameters of radicals generated in waxy maize and in Hylon VII at 210 and 230 °C

Sample	210 °C			230 °C		
	Signal I	Signal II	Signal III	Signal I	Signal II	Signal III
Native waxy maize	Content 30% rhombic $g_z = 2.002$ $g_x = 2.005$ $g_y = 2.010$ $g_{av} = 2.006$ ($s_H = ½$) $A_z = 0.9$ mT $A_x = 1.1$ mT $A_y = 1.3$ mT $A_{av} = 1.1$ mT	Content 70% rhombic $g_z = 2.004$ $g_x = 2.006$ $g_y = 2.010$ $g_{av} = 2.007$	–	Content 19% rhombic $g_z = 2.006$ $g_x = 2.002$ $g_y = 2.010$ $g_{av} = 2.006$ ($s_H = ½$) $A_z = 1.3$ mT $A_x = 0.8$ mT $A_y = 1.4$ mT $A_{av} = 1.2$ mT	Content 81% rhombic $g_z = 2.003$ $g_x = 2.006$ $g_y = 2.009$ $g_{av} = 2.006$	–
Pressurized waxy maize	Content 17% rhombic $g_z = 2.002$ $g_x = 2.006$ $g_y = 2.010$ $g_{av} = 2.006$ ($s_H = ½$) $A_z = 0.9$ mT $A_x = 1.3$ mT $A_y = 1.4$ mT $A_{av} = 1.2$ mT	Content 83% rhombic $g_z = 2.003$ $g_x = 2.006$ $g_y = 2.010$ $g_{av} = 2.006$	–	Content 7% rhombic $g_z = 2.002$ $g_x = 2.004$ $g_y = 2.005$ $g_{av} = 2.004$ ($s_H = ½$) $A_z = 0.9$ mT $A_x = 1.0$ mT $A_y = 0.9$ mT $A_{av} = 0.9$ mT	Content 93% rhombic $g_z = 2.002$ $g_x = 2.005$ $g_y = 2.008$ $g_{av} = 2.005$	–
Native Hylon VII	Content 26% rhombic $g_z = 2.003$ $g_x = 2.004$ $g_y = 2.016$ $g_{av} = 2.008$ ($s_H = ½$) $A_z = 1.3$ mT $A_x = 1.0$ mT $A_y = 2.0$ mT $A_{av} = 1.4$ mT	Content 74% rhombic $g_z = 2.004$ $g_x = 2.005$ $g_y = 2.005$ $g_{av} = 2.005$	–	–	Content 100% rhombic $g_z = 2.003$ $g_x = 2.006$ $g_y = 2.006$ $g_{av} = 2.005$	–
Pressurized Hylon VII	–	Content 71% rhombic $g_z = 2.008$ $g_x = 2.011$ $g_y = 2.005$ $g_{av} = 2.008$	Content 29% rhombic $g_z = 2.003$ $g_x = 2.003$ $g_y = 2.004$ $g_{av} = 2.003$	–	Content 83% rhombic $g_z = 2.005$ $g_x = 2.008$ $g_y = 2.002$ $g_{av} = 2.005$	Content 17% rhombic $g_z = 1.991$ $g_x = 2.001$ $g_y = 2.002$ $g_{av} = 1.998$

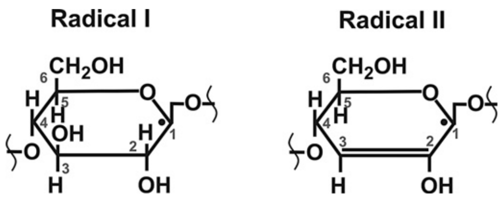

Fig. 6. Radicals generated thermally in waxy maize starch and in Hylon VII by abstraction of hydrogen ($H^{\cdot}$) from glucose unit at C_1 atom (Radical I) or by additional dehydration at C_2 and C_3 atoms (Radical II).

The most significant effects differentiating waxy maize starch from Hylon VII consist on higher number of radicals generated at 210–230 °C in non-pressurized waxy maize starch and more effective reduction of this number in waxy maize starch pretreated with high hydrostatic pressure. It should be noticed, that both types of the investigated starches may content small amounts of proteins and lipids and that amount of these impurities is usually different for waxy maize and Hylon VII. For instance, according to Morrison (1988) content of lipids in amylose-rich samples (Hylon VII) is higher (964 mg/100 g of the starch) than in waxy maize (80 mg/100 g of the starch), while according to Debet & Gidley, 2006 content of nitrogen, which is a measure of the amount of proteins, in Hylon VII is also higher (106 mg/g) than in waxy maize starch (24 mg/g). Taking into account that the observed effects of thermal and high pressure treatment on the number of radicals generated in waxy maize starch, are – in spite of smaller amount of impurities – more distinct than in Hylon VII we conclude that these additives do not influence significantly the EPR data. This conclusion is in line with results obtained by Ciesielski et al. (1997) for cereal flours containing various amount of proteins and lipids where no effect of these impurities on thermal generation of radicals was found.

3.3. Radicals generated in the presence of spin trap

To check, if in waxy maize starch and in Hylon VII, besides relatively stable thermally generated radicals, some short living radicals are formed, the thermal and high pressure treatment were performed also in the presence of a PBN spin trap (Fig. 7). In this case, in order to avoid thermal decomposition of PBN, the samples were heated at relatively low temperature (180 °C). In a blank experiment it was found that the contribution of paramagnetic species generated in PBN itself during heating at 180 °C to the total signal intensity was negligible (smaller than 0.07%).

In the presence of PBN the waxy maize starch heated at 180 °C shows an EPR signal with two components find by simulation

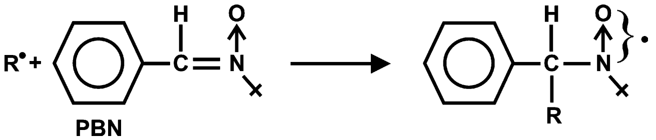

Fig. 7. Reaction of the *N-tert*-butyl-α-phenylnitrone (PBN) with a short living radical $R^{\cdot}$ generated thermally in amylose and/or in amylopectin at 180 °C in air.

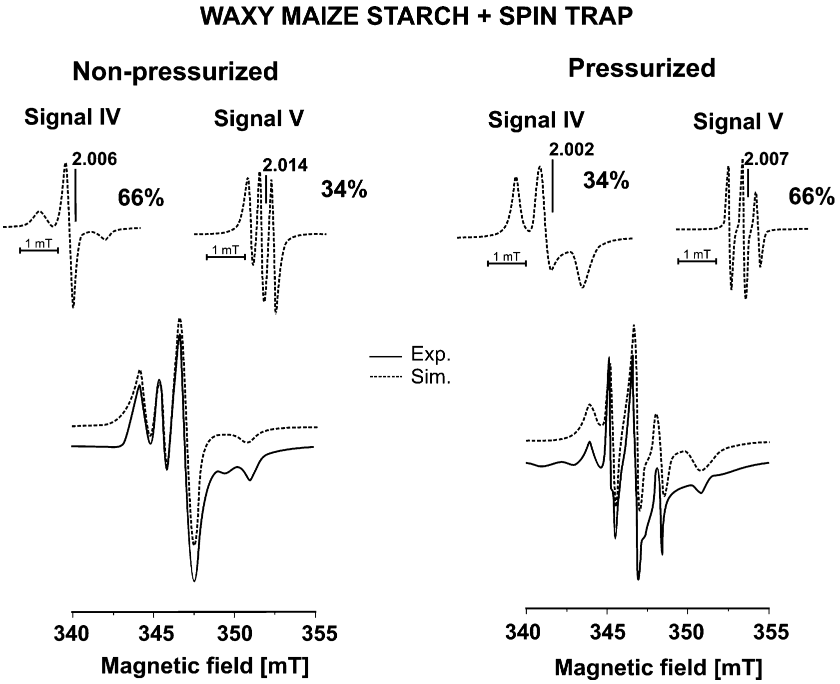

Fig. 8. Effect of high pressure treatment on the shape of EPR signals (registered at 20 °C) of radicals generated in waxy maize starch by heating at 180 °C in the presence of spin trap (PBN).

Table 2
EPR parameters of radicals generated at 180 °C in waxy maize and in Hylon VII in the presence of spin trap (PBN)

Sample	Signal IV		Signal V
Native waxy maize + PBN	Content: 66% rhombic		Content: 34% isotropic
	$g_z = 2.004$		$g_{iso} = 2.014$
	$g_x = 2.007$	($s_N = 1$)	($s_N = 1$)
	$g_y = 2.006$	$A_{av} = 1.3$ mT	$A_{iso} = 1.2$ mT
	$g_{av} = 2.006$		
Pressurized waxy maize + PBN	Content: 34% rhombic		Content: 66% isotropic
	$g_z = 2.005$		$g_{iso} = 2.007$
	$g_x = 1.990$	($s_N = 1$)	($s_N = 1$)
	$g_y = 2.010$	$A_{av} = 1.3$ mT	$A_{iso} = 1.5$ mT
	$g_{av} = 2.002$		
Native Hylon VII + PBN	Content: 78% rhombic		Content: 22% isotropic
	$g_z = 2.004$		$g_{iso} = 2.015$
	$g_x = 2.008$	($s_N = 1$)	($s_N = 1$)
	$g_y = 2.008$	$A_{av} = 1.3$ mT	$A_{iso} = 1.2$ mT
	$g_{av} = 2.006$		
Pressurized Hylon VII + PBN	Content: 50% rhombic		Content: 50% isotropic
	$g_z = 2.005$		$g_{iso} = 2.013$
	$g_x = 2.006$	($s_N = 1$)	($s_N = 1$)
	$g_y = 2.003$	$A_{av} = 1.6$ mT	$A_{iso} = 1.2$ mT
	$g_{av} = 2.005$		

(Fig. 8 and Table 2). The total intensity of the spectrum, equal to 10^{16} spins/g, is of one order of magnitude higher than that observed in the absence of PBN (Figs. 4 and 10) indicating formation of short living paramagnetic species stabilized by the spin trap. The dominating component signal IV (66%) with rhombic symmetry and $g_{av} = 2.006$ and isotropic signal V with $g_{iso} = 2.014$ exhibit hyperfine structure (HFS). The three-line HFS with $A_{av} = 1.3$ mT and $A_{iso} = 1.2$ mT for signals IV and V, respectively, indicates interaction of the unpaired electron with nuclear spin of nitrogen ($I = 1$) in PBN-starch adducts (Fig. 7). Signal IV is anisotropic, which means partial immobilization of the adduct, while signal V is isotropic, probably due to its effective rotation, resulting in averaging of the signal anisotropy. Comparison of the shape of the signals IV and V with the published data (Jeschke & Schlick, 2006) leads to the conclusion, that the rotational correlation times of radical adducts with signal IV and V are of the order 10^{-6} and 10^{-10} s, respectively. The presence of two types of rotating species with different rotational correlation time indicates, according to Kruczala, Varghese, Bokria, and Schlick (2003), that the spin adducts are located in two different parts of the amorphous phase: one dynamically restricted by the proximity to the crystalline phase (slow rotating component) and another one, free of any restriction (fast rotating component).

The pressurized waxy starch reveals after heating at 180 °C, in the presence of PBN, the EPR spectrum with much sharper component lines than before pressurization (Fig. 8). Two components of the spectrum may be distinguished, however, the fast rotating species is now dominating (66%). According to the concept of the

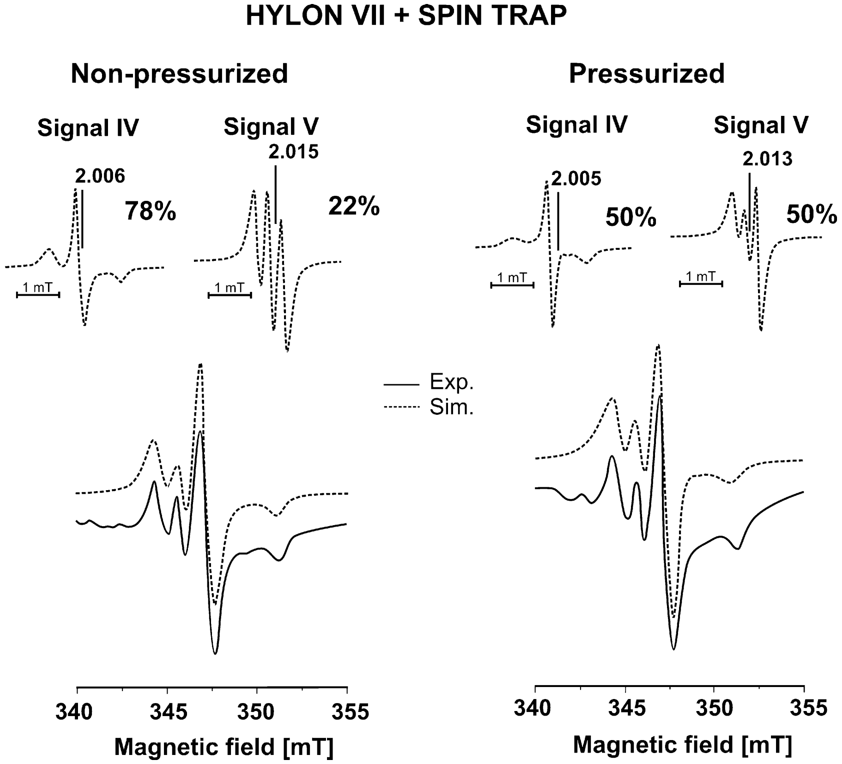

Fig. 9. Effect of high pressure treatment on the shape of EPR signals (registered at 20 °C) of radicals generated in Hylon VII by heating at 180 °C in the presence of spin trap (PBN).

dynamically restricted amorphous phases (Kruczala et al., 2003) this result indicates that upon high pressure the crystallinity of the waxy maize starch diminishes, which in fact was evidenced experimentally (Błaszczak et al. 2005a, b; Katopo et al., 2002). This effect makes amorphous domains, unrestrained in their dynamics by the proximity of crystal regions, dominating.

The isotropic component (signal V) with higher A_{iso} value (A_{iso} = 1.5 mT) than that observed for non-pressurized samples reveals stronger interaction of unpaired electron with the nuclear magnetic moment of nitrogen, which means shortening of distances in the PBN adduct.

The native sample of Hylon VII heated with PBN at 180 °C exhibits, similarly to waxy maize starch, a two component EPR spectrum (Fig. 9 and Table 2) with dominating (78%) signal IV. After pressurization the contribution of fast rotating component, with signal V, increases, the later effect is, however, less significant for Hylon VII than for waxy maize starch. These finding corroborates with previously published data concerning higher susceptibility to high pressure treatment of the starch containing mostly amylopectin than that enriched in amylose (Błaszczak et al. 2005a, b; Błaszczak, Fornal, et al., 2007; Błaszczak, Misharina, et al., 2007; Katopo et al., 2002).

In the case of both types of the starch (waxy maize and Hylon VII) the total intensity of the EPR spectra significantly diminishes for the pressurized samples (Fig. 10). It may be supposed, that the reduction of the number of radicals is due, similarly as in the case of radicals generated in the absence of PBN, to the water molecules squeezing into the starch granules upon high hydrostatic pressure.

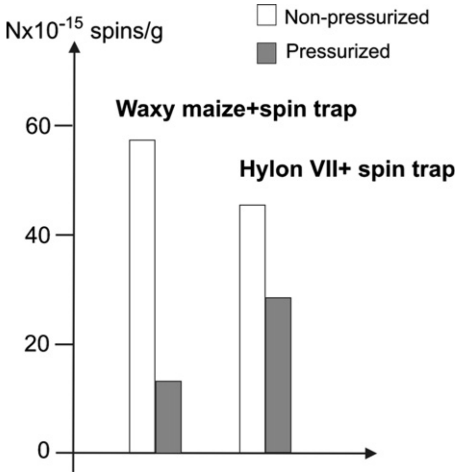

Fig. 10. Effect of high pressure treatment on the number of radicals generated in waxy maize starch and Hylon VII by heating at 180 °C in the presence of spin trap (PBN).

4. Conclusions

High pressure has a significant influence on radical processes in waxy maize starch and in Hylon VII. Various paramagnetic species are generated upon thermal treatment of the native and previously pressurized samples. These are short living radicals formed at relatively low temperature (180 °C), stabilized by a spin trap (PBN), and long living radicals generated at higher temperatures (210–230 °C).

Content of water and high pressure treatment have a significant influence on the number of radicals generated thermally in both types of the investigated starches. In the presence of water the annihilation of radicals is accelerated, the effect being more significant upon high pressure treatment.

The EPR spectra of short living radicals, stabilized by a spin trap, reveal the presence of slow and fast rotating species. The relative content of radicals with the short rotational correlation time considerably increases after high pressure treatment. This effect is related with the decrease of crystallinity of the starch matrix, especially pronounced in the case of waxy maize.

The obtained results indicate that effective reduction of the number of radicals generated thermally in starch at temperatures commonly used for preparing food may be achieved by pressurization of the starch in the presence of water.

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