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EPR study of the influence of high hydrostatic pressure on the formation of radicals in phosphorylated potato starch

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ABSTRACT

Phosphorylated potato starch reveals smaller values of the melting enthalpy and lower crystallinity than the native one, indicating weakening of the structure upon incorporation of phosphorus. Nevertheless, changes in transition temperatures T_o and T_p suggest that certain cross-linking reactions occur in the case of distarch phosphates. Heating at 210–230 °C causes easier generation of radicals in phosphorylated than in the native starch. Pretreatment of the phosphorylated starch with high hydrostatic pressure somewhat diminishes the number of thermally generated radicals. Both effects, increase in the number of radicals upon phosphorylation and its decrease upon pretreatment of the starch with pressure, are more pronounced for monostarch than for distarch phosphates, most probably due to the cross-linking reactions occurring in the distarch phosphate samples.

1. Introduction

Starch plays an important role not only in producing formulated functional foods but most of all in enhancing functionality of food products [Yuryev, Wasserman, Andreev, & Tolstoguzov, 2002, chap. 2]. Differences in functional properties (e.g. rheological stability, susceptibility to chemical, physical, enzymatic treatment) between starches can be directly assigned to the structural organization of granule, i.e. amylose concentration, degree of polymerization, content of amylose/amylopectin defects in crystalline/amorphous lamellae, chain-length distribution of amylopectin within lamellae and/or amylopectin clusters, crystallinity type, etc. Modification of starch structure by mentioned treatments involves the changes in physicochemical properties of the biopolymer which in turn results in improving its functional characteristics by means of, e.g. alteration in gelatinization and pasting behaviour [Singh, Kaur, & McCarthy, 2007]. These changes – triggered by modification – can be studied in relation to thermodynamic and functional properties of the starch using differential scanning calorimetry (DSC). The calorimetric data might provide information about differences in structure between starches of various botanical origin as well as between native and modified samples. It was found, e.g. that defects within crystalline lamellae (e.g. disordered ends of amylopectin double helices, the helices unpacked inside crystallities, amylose tie-chains) and also amylose-lipid complexes had destabilizing effect on structure organization of the lamellae resulting in a decrease of their melting temperature [Koroteeva et al., 2007]. The melting temperature was considered as a function of the starch polymorphous structure and thickness of crystalline lamellae while the values of melting enthalpy were found to be proportional to the starch crystallinity [Bocharnikova et al., 2003].

An important method of chemical modification of the starch is phosphorylation. Special attention is given to physiological effects of phosphorylated starch used in human diet. The food-grade resistant starch, recommended for daily intake at a level of 20–30 g by various Diabetes Associations and Cancer Institutes [Ohr, 2004], may be produced by substitution of OH^- with PO_4^{3-} groups and formation of mono- or distarch phosphates [Lim & Seib, 1992; Sang & Seib, 2006]. Such modifications – beneficial for diet – may, however, change the ability of starch to be thermally degraded. It is well known that such degradation is accompanied by formation of free radicals, which are not indifferent for human health. Evidences are given in literature that some radicals may cause serious diseases [Steinberg, 1995] and accelerate negative cellular changes associated with aging [Ashok & Ali, 1999].

The objective of the present work was to determine the main factors controlling the amount and properties of radicals formed in the phosphorylated starch during heat treatment at temperatures usually applied for preparing food. The influence of the pretreatment of the modified starch with high hydrostatic pressure on radical formation was also tested. The aim was to check if the recently discovered effect of reduction the number of radicals upon pres-

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surization of the native starch [Błaszczak, Bidzińska, Dyrek, Fornal, & Wenda, 2008] is operating also in the case of phosphorylated samples. Differential scanning calorimetry and quantitative electron paramagnetic resonance (EPR) spectroscopy were applied to study these processes.

2. Materials and methods

2.1. Materials

Native potato starch purchased from Sigma (S-9679) was used for phosphorylation performed according to Lim and Seib (1992) and Sang and Seib (2006).

2.1.1. Monostarch phosphate

Starch (10 g) was added gradually to the aqueous solution (14 mL) containing 10.5% (starch base) of sodium trimetaphosphate/sodium tripolyphosphate (STMP/STPP, 99/1, w/w) and 0.5 g Na_2SO_4 at initial pH = 6 (obtained with 0.2 M HCl). The whole suspension was then mixed during 60 min at room temperature. Afterwards the slurry was evaporated at temperature 45–50 °C until content of water was equal to 15–20%. The obtained material was heated at 130 °C for 2 h. At these conditions the most effective incorporation of phosphorus into the starch structure took place [Sang & Seib, 2006]. After cooling the cake was mixed with distilled water (70 mL) and pH was measured. Then the suspension was centrifuged and 120 mL of distilled water was added to the solid residue. After mixing the pH value was adjusted to 6.5 with 0.2 M solution of NaOH. Washing and centrifugation was repeated three times and finally the product of phosphorylation was dried at 50 °C during 20 h.

2.1.2. Distarch phosphate

Similar procedure [Lim & Seib, 1992; Sang & Seib, 2006] was applied to obtain distarch phosphate with the only difference consisting on the initial value of pH = 11 (obtained with 0.2 M NaOH) of the STMP/STPP solution used for phosphorylation of the starch.

2.1.3. Reference samples

Reference samples were obtained by the same procedure as applied for starch transformed into mono- and distarch phosphates but without adding the phosphorylating agents (STMP, STPP). These samples were used to extract the net effect of phosphorus from the DSC and EPR data.

2.2. Methods

2.2.1. Determination of phosphorus content

Total phosphorus content was determined according to Polish norm PN-EN ISO 3946. Samples of phosphorylated starch were mineralized with the concentrated nitric and sulphuric acids. Ortophosphoric acid formed during mineralization reacted with ammonium molybdate $[(NH_4)_6Mo_7O_{24}{\cdot}4H_2O]$ giving a complex of molybdenum (VI) ($[P^{V}Mo^{VI}{}_{12}O_{40}]^{3-}$), which afterwards was reduced with ascorbic acid to a deep blue complex of Mo(V). The absorbance of the blue solution was measured at wavelength 680 nm with a Spektralphotometer SPECOL 11 produced by C. Zeiss (Jena, Germany). The content of phosphorus was determined by comparing the absorbance values of the investigated solutions with calibration curves. The maximum error of determination, equal to 10%, was calculated as a standard deviation from 3 to 4 independent analytical results.

2.2.2. Pressure treatment

Pressure treatment was performed using 30% (w/v) starch-water suspensions closed in Teflon tubes (10 mL), precisely mixed, deaerated and sealed [Błaszczak, Valverde, & Fornal, 2005]. For high pressure treatment a press type LV30/16, produced by The Centre of High Pressure Analysis, Polish Academy of Sciences, Warsaw, Poland was used. The Teflon tubes were put into a high pressure chamber (with the capacity of approximately 25 mL), filled with pressure-transmitting medium, which also minimalized 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 50 °C to constant weight. The dry samples were pulverized with an agate mortar.

The non-phosphorylated potato starch samples, used as references, and phosphorylated ones, were treated in a similar way.

2.2.3. Thermal treatment

Starch samples of about 30 mg were placed in EPR quartz tubes (inner diameter = 3 mm), heated in an oven for 30 min at 150 °C and afterwards for 30 min at 210 °C or 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.

2.2.4. Differential scanning calorimetry

The DSC analyses were performed in a differential scanning calorimeter equipped with an Intra-cooling system (Diamond DSC AS, PerkinElmer). The amount of sample used for measurement was 10 mg with the addition of 25 μL of water. The samples were sealed in stainless steel pans, equilibrated for 2 h, and scanned. The heating rate was 10 °C/min over the temperature range 20–110 °C. A 25 μL quantity of water was used as a reference to counterbalance the mass of water on the sample side.

The values of the gelatinization degree (GD) were calculated using the following equation:

$$GD = \{(\Delta H_{ns} - \Delta H_{ts})\Delta H_{ns}^{-1}\} \times 100\%,$$

where ΔH_{ns} and ΔH_{ts} are the melting enthalpies of native and treated starches, respectively.

Calculation of the percent of crystallinity of analyzed starches was performed according to the Temperature Dependent Crystallinity DSC software offered by PerkinElmer.

2.2.5. Light microscopy (LM) studies

The starch powders were suspended in a drop of water on a microscopic glass, covered by cover slip, and they were observed under optical microscope (OLYMPUS BX60) using polarized light.

2.2.6. 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. $VOSO_4{\cdot}5H_2O$ diluted with diamagnetic K_2SO_4, containing 5×10^{19} spins/g, was used as a primary standard. All necessary precautions, discussed in papers [Dyrek, Madej, Mazur, & Rokosz, 1990; Dyrek, Rokosz, & Madej, 1994] were followed in order to assure good precision of the quantitative EPR measurements. Generation of radicals was investigated on native and phosphorylated samples before and after pressurization and heat treatment.

EPR parameters of the radicals were determined by a simulation procedure using the program EPR SIM 32 [Spałek, Pietrzyk, & Sojka, 2005]. The accuracy of determination of g values was ±0.001 and that of A values was ±0.5 G.

Table 1
The values of transition temperatures (T_o, T_p), melting enthalpies (ΔH), crystallinity and degree of gelatinization (GD) of native and treated starches.

	T_o (°C)	T_p (°C)	ΔH (J/g)	GD (%)	Crystallinity (%)
Before high pressure treatment					
Native potato starch	62.72	67.03	16.48		25
Reference sample of monostarch	67.44	70.25	12.94	21	17
Reference sample of distarch	64.37	67.09	11.99	27	16
Monostarch phosphate	51.84	59.06	3.68	78	4
Distarch phosphate	65.04	68.08	1.20	93	2
After treatment with high pressure					
Potato starch	61.77	68.00	9.57	42	12
Reference sample of monostarch	63.50	66.58	9.41	43	10
Reference sample of distarch	59.37	65.03	8.46	49	12
Monostarch phosphate	67.59	70.99	0.66	96	3
Distarch phosphate	61.15	66.63	0.30	100	Amorphic

3. Results and discussion

3.1. Influence of phosphorylation and/or pressure treatment on thermodynamic parameters and morphology of starches

DSC studies demonstrated that chemical modification (phosphorylation) of potato starch significantly altered its thermodynamic parameters, i.e. transition temperatures (T_o, T_p) and melting enthalpy (ΔH) (Table 1). A significant decrease in melting enthalpy observed in the case of phosphorylated starches indicated that phosphate substitution reduced crystalline regions within granules. Singh et al. (2007) reported that values related to T_p and ΔH can be assigned to a measure of crystalline quality (double helix length) and an overall measure of starch crystallinity (quantity and quality), respectively. Crystallinity values calculated for starch phosphates demonstrated the loss of molecular order, which resulted in advanced and/or complete gelatinization (Table 1). For monostarch phosphate lower values of transition temperatures (T_o and T_p) in comparison to native starch were observed, whereas for distarch phosphate the values of T_o and T_p were slightly higher than those for native sample (Table 1). An opposite relation between melting enthalpy and transition temperatures obtained for distarch phosphate suggests, that some cross-linking reaction might occur on treatment of potato starch with STMP/STPP mixture in the alkaline medium. An increase in gelatinization temperature was already reported in literature for cross-linked starches [Sang & Seib, 2006; Singh et al., 2007] the effect being strongly dependent on the concentration and type of cross-linking reagent as well as on reaction conditions. The opposite effect of enthalpy (ΔH) and transition temperatures (T_o and T_p), indicating occurrence of cross-linking, was observed only for distarch phosphates obtained in alkaline medium and not for monostarch phosphates prepared in acidic conditions (Table 1). In both cases the same reagents (STMP/STPP, 99/1, w/w) were used. This might indicate that the main factor controlling appearance or lack of the cross-linking during phosphorylation was the pH value.

The opposite relation between enthalpy and transition temperatures was also observed in the case of reference potato starches (treated as starch phosphates but non-phosphorylated) (Table 1) but for reasons quite different than those operating in the phosphorylated starches. The effect is significant only for reference of monostarch phosphate, obtained in acidic medium, whereas for reference of distarch specimen (starting pH value equal to 11) it is only slightly higher than the experimental error. It is known, that in acidic medium the starch hydrolysis occurs preferentially on amorphous material [Komiya, Yamada, & Nara, 1987; Tomasik & Schilling, 2004], leading to the increase in the relative content of the crystalline phase. This phase exhibits higher T_o and T_p values, i.e. is less receptive to gelatinization, which justifies the observed effect. It may be thus concluded that the thermodynamic parameters of starches obtained from calorimetric data were influenced by the pH values at which modification of the starch (hydrolysis or phosphorylation) takes place.

All the analyzed starches treated with high hydrostatic pressure presented lower values of melting enthalpy, lower crystallinity, and higher values of gelatinization degree compared to non-pressurized samples (Table 1). The lowest values of melting enthalpy and the highest percent of gelatinized granules revealed the pressurized monostarch and distarch phosphates. These results indicate that the combined treatment of granules with high pressure and phosphorylation result in their effective disordering and – in the case of distarch phosphate – in formation of a completely amorphous material.

The significant effect of chemical modification and high pressure treatment on granule crystallinity was also observed on presented LM micrographs (Fig. 1A–J). The non-pressurized granules of native potato starch (Fig. 1A) and reference samples (Fig. 1C and E), demonstrate Maltese cross under polarized light, due to their birefringence resulting from radial orientation of the crystallites formed by double helices of polysaccharide chains [Yuryev et al., 2002, chap. 2].

The phosphorylated non-pressurized starches revealed advanced loss of birefringence (Fig. 1G and I). That fact confirmed the above expressed suggestion that phosphorylation led to the distinct alteration of the granule structure.

Treatment of non-phosphorylated starch granules with high pressure in an excess of water affected the ordered state of the crystallites, which elicited visible granules swelling and deformation of Maltese cross (Fig. 1B, D and F). These observations are consisted with studies devoted to high pressure treated starches presented in literature [Błaszczak, Valverde, & Fornal, 2005; Błaszczak, Fornal, Valverde, & Garrido, 2005; Stolt, Oinonen, & Autio, 2001].

In the case of phosphorylated and afterwards pressure-treated starches a disappearance of the Maltese cross was found (Fig. 1H and J). A lack of birefringence resulted directly from their amorphous character.

3.2. Influence of phosphorylation on the number of radicals

The phosphorylated potato starch did not show any EPR signal before thermal treatment. However, after heating at temperatures conventionally used for preparing of the food products (210–230 °C) in the EPR spectra of both: monostarch and distarch phosphates two component signals appear, similar to those observed for native potato starch [Dyrek et al., 2007]. The EPR signal of radical I (Fig. 2), generated by abstraction of hydrogen

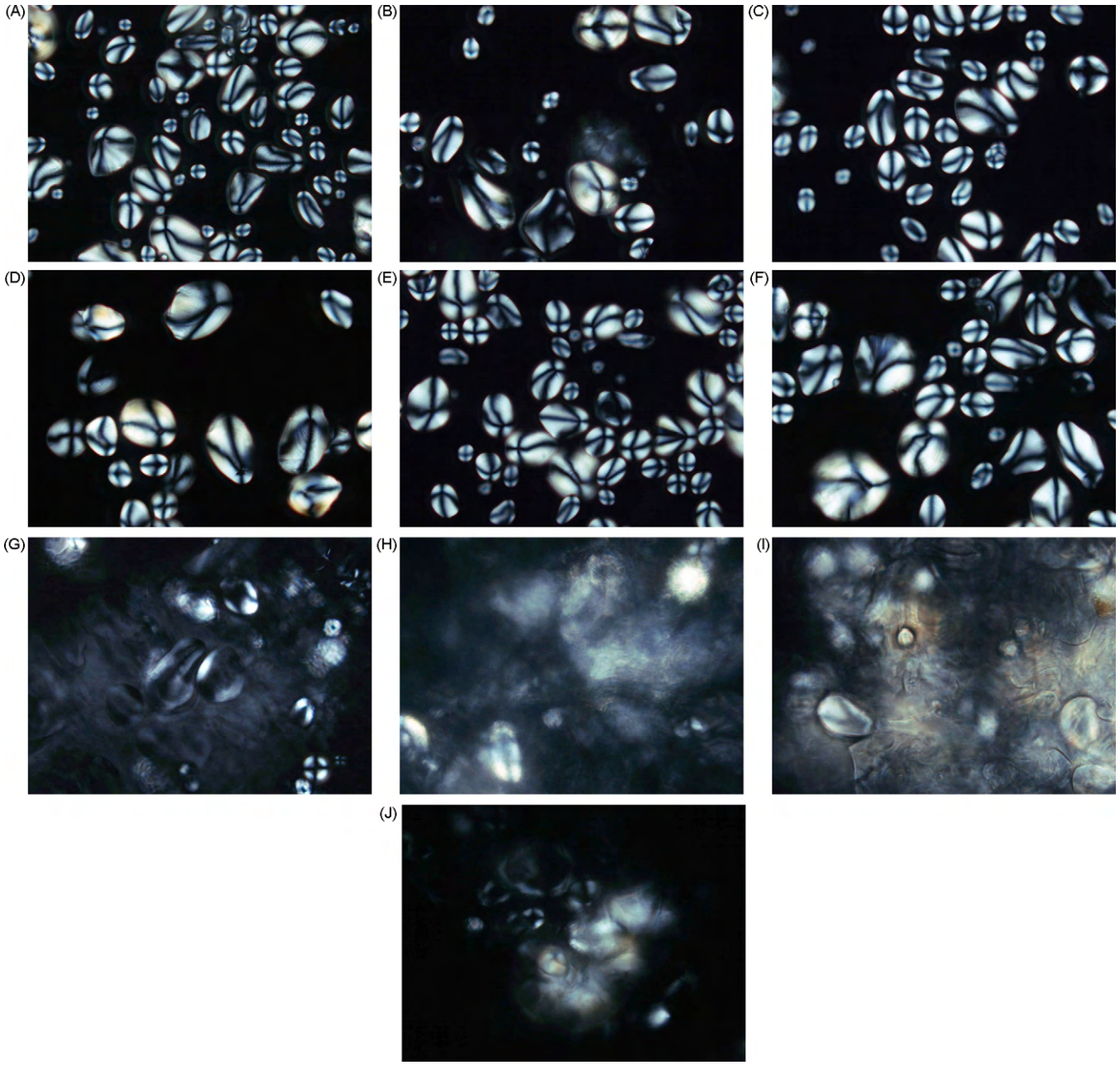

Fig. 1. Polarized light micrographs of native potato starch (A, non-press.; B, press.); reference sample of monostarch phosphate (C, non-press.; D, press.); reference sample of distarch phosphate (E, non-press.; F, press.); monostarch phosphate (G, non-press.; H, press.); distarch phosphate (I, non-press.; J, press.). The B, D, F, H, and J micrographs presented starches after high pressure treatment at 650 MPa/9 min.

($H^{\bullet}$) from C-1 atom of the glucose unit, exhibits hyperfine structure (HFS), indicating interaction of the unpaired electron localized at C-1 with nuclear spin of hydrogen (localized at C-2). The EPR signal of the radical II, without HFS, represents carbon radical not interacting with neighboring hydrogen. The formation of this radical requires simultaneous abstraction of hydrogen and dehydration (Fig. 2).

The number of radicals generated after heating at 210 °C or 230 °C of the native and phosphorylated potato starch is presented in Fig. 3, where the values of experimental error are marked by bars equal to the standard deviation of 2 to 6 independent measurements. The smallest number of radicals was generated at both temperatures in the native potato starch containing about 0.05% of phosphorus. Under the influence of phosphorylation the number of radical species considerably increases, indicating that incorporation of phosphorus into the starch in the form of both: monostarch and distarch phosphates destabilizes its structure. The reference samples, obtained by treatment of the starch in the same way as required by phosphorylation procedure, but without adding phosphorus compounds, show the number of radicals intermediate between native and phosphorylated starch. This means that upon heating of the starch without phosphorylating agent at 45–130 °C in alkaline (distarch procedure) or acidic (monostarch procedure) medium [Lim & Seib, 1992; Sang & Seib, 2006] hydrolysis of the starch occurs [Tomasik & Schilling, 2004] leading to the efficient weakening of the structure.

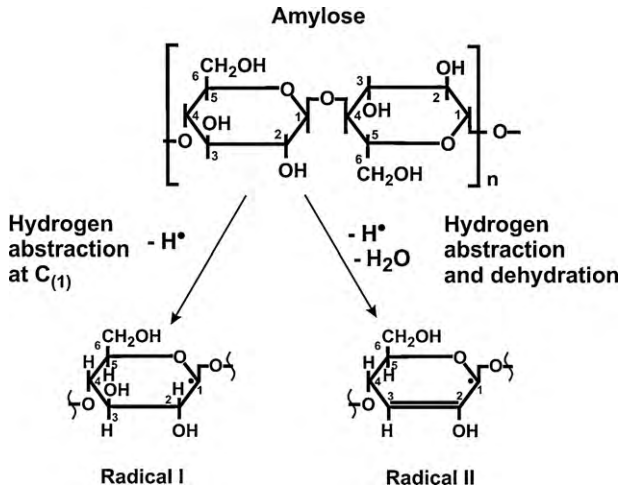

Fig. 2. Mechanism of formation radicals during thermal treatment of the starch [Dyrek et al., 2007].

After treatment of the phosphorylated samples at 210 °C the increase in the number of radicals is more pronounced for monostarch (in spite of smaller content of phosphorus equal to 0.60% P) than for distarch phosphates (containing 0.75% P). This effect may be related with some cross-linking occurring – as suggested by calorimetric data – in distarch phosphate, which makes generation of radicals more difficult.

After heating at 230 °C two types of behaviour may be distinguished (Fig. 3). The samples with small contents of phosphorus (native starch and reference samples) exhibited almost the same number of radicals which was about twice smaller than those generated in chemically phosphorylated potato starch. At 230 °C the equalization of the number of radicals occurs also in the phosphorylated samples which indicates, that at this temperature dominating role in generation of radicals plays thermal activation, whereas the influence of the type of bonding phosphorus (mono- or distarch) is less pronounced.

3.3. Influence of pressure on the number of radicals

In the case of pressurized monostarch phosphates thermally treated at 210 °C smaller increase in the number of radicals was

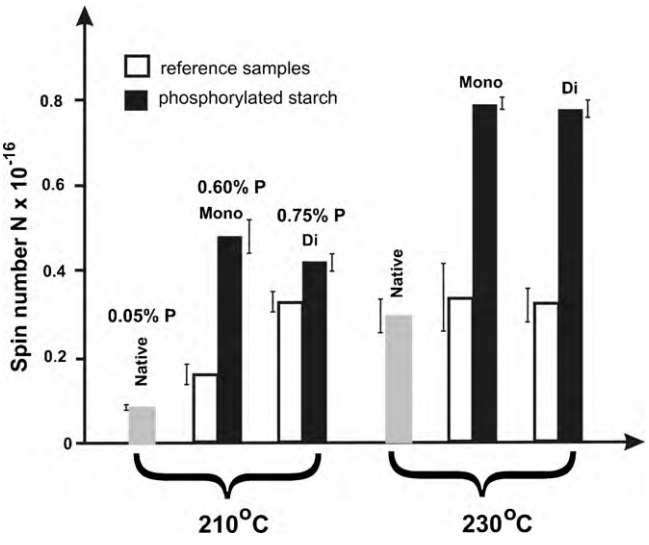

Fig. 3. Influence of temperature on the number of radicals generated by heating of the phosphorylated potato starch.

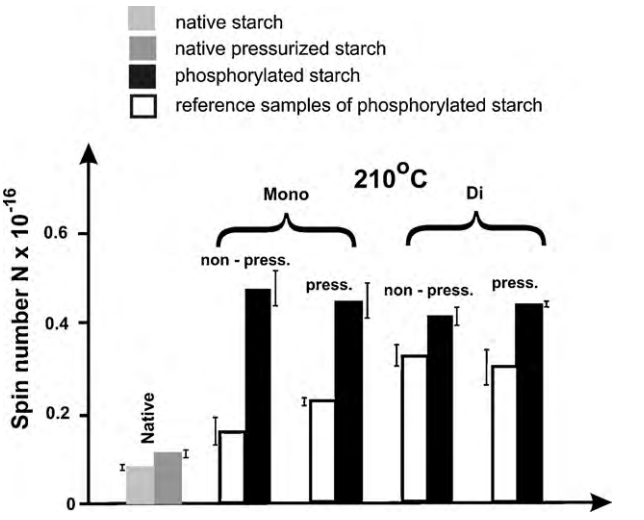

Fig. 4. Influence of pressurization on the number of radicals generated by heating at 210 °C of the phosphorylated potato starch.

observed than in the non-pressurized samples (Fig. 4). For distarch phosphates the effect of pressure was almost insignificant.

In our previous paper [Błaszczak et al., 2008] we presented results concerning reduction of the number of thermally generated radicals in corn starch pretreated with high hydrostatic pressure. In the present work an opposite effect of phosphorylation was found (Fig. 3), i.e. the increase of the number of radicals created in the samples containing phosphorus. Evidently, in phosphorylated and pressurized potato starch the two factors: phosphorylation and pretreatment with high pressure act in opposite direction blurring the ultimate effect of the change in the number of radicals.

3.4. Influence of phosphorylation and pressure on EPR parameters of radicals

The radicals generated by heating of the native and phosphorylated potato starch before and after pressurization (Tables 2 and 3; Fig. 5) give at room temperature EPR spectra with two components: signal of radical I and signal of radical II (Fig. 2). Under the influence of phosphorylation the content of radical I generated at both temperatures (210 °C and 230 °C) in non-pressurized samples (Table 2) decreases. Simultaneously, the values of g factor of both components (I and II) decrease, indicating smaller influence of oxygen of the glucose units on the character of radicals generated in phosphorylated samples, comparing with the native starch.

The pretreatment of potato starch with high hydrostatic pressure (Table 3) induces an opposite effect: the content of radical I increases and *g* factor values of both components (I and II) also increase compared with non-pressurized samples. The first result may be caused by water molecules squeezing into the starch granule upon the high hydrostatic pressure and preventing dehydration, i.e. abstraction of hydrogen from C-2 and OH group from C-3 of the glucose unit (Fig. 2). That conclusion is supported by a compression phenomenon occurring under hydrostatic pressure at which water volume is compressed up to 15% [Trejo Araya et al., 2007]. According to this suggestion the water molecules may penetrate internal structure of granules decreasing their crystallinity degree [Błaszczak et al., 2007; Błaszczak, Valverde, & Fornal, 2005; Błaszczak, Fornal, Valverde, & Garrido, 2005; Katopo & Jay-lin, 2002]. Trejo Araya et al. (2007) paid also attention to a fact that during compression certain pressure threshold appeared above which the structure of treated product might not be further compressed or be partially/completely disrupted.

Table 2
EPR parameters of radicals generated by heating of the phosphorylated non-pressurized potato starch.

Sample	Temperature of treatment [°C]	Radical I						Radical II		
		Content [%]	A [G]		g		ΔB_{av} [G]	g		ΔB_{av} [G]
Native potato starch	210	**33**	8.60 14.47 14.81	**12.6**	2.0043 2.0071 2.0102	**2.0072**	**3.2**	2.0046 2.0064 2.0101	**2.0070**	**4.4**
	230	**26**	10.05 14.62 14.92	**13.2**	2.0061 2.0063 2.0101	**2.0075**	**2.8**	2.0045 2.0071 2.0099	**2.0072**	**4.9**
Reference of monostarch phosphate	210	**23**	12.25 12.51 13.24	**12.7**	2.0029 2.0053 2.0093	**2.0058**	**3.2**	2.0026 2.0057 2.0091	**2.0058**	**4.6**
	230	**16**	12.50 13.19 15.83	**13.8**	2.0051 2.0025 2.0083	**2.0053**	**3.1**	2.0021 2.0050 2.0087	**2.0053**	**5.4**
Monostarch phosphate	210	**20**	11.96 11.98 15.54	**13.2**	2.0032 2.0053 2.0087	**2.0057**	**3.3**	2.0031 2.0054 2.0091	**2.0059**	**5.0**
	230	**12**	13.57 9.89 14.11	**12.5**	2.0025 2.0055 2.0059	**2.0046**	**4.9**	2.0022 2.0037 2.0067	**2.0042**	**6.0**
Reference of distarch phosphate	210	**35**	11.68 14.01 16.97	**14.2**	2.0045 2.0065 2.0095	**2.0068**	**2.7**	2.0044 2.0063 2.0098	**2.0068**	**3.7**
	230	**17**	10.85 13.00 14.00	**12.6**	2.0024 2.0052 2.0090	**2.0055**	**3.6**	2.0022 2.0053 2.0091	**2.0055**	**4.9**
Distarch phosphate	210	**26**	12.00 12.47 16.03	**13.5**	2.0029 2.0051 2.0084	**2.0055**	**3.2**	2.0041 2.0045 2.0083	**2.0056**	**5.5**
	230	**15**	12.69 11.36 12.92	**12.3**	2.0040 2.0045 2.0062	**2.0049**	**5.8**	2.0049	**2.0049**	**7.0**

Table 3
EPR parameters of radicals generated by heating of the phosphorylated pressurized potato starch.

Sample	Temperature of treatment [°C]	Radical I						Radical II		
		Content [%]	A [G]		g		ΔB_{av} [G]	g		ΔB_{av} [G]
Native potato starch	210	**42**	9.02 12.94 16.56	**12.8**	2.0054 2.0065 2.0101	**2.0073**	**2.2**	2.0056 2.0081 2.0111	**2.0083**	**5.9**
	230	**33**	9.09 11.93 15.71	**12.2**	2.0048 2.0064 2.0100	**2.0071**	**1.9**	2.0049 2.0070 2.0102	**2.0074**	**5.0**
Reference of monostarch phosphate	210	**31**	11.31 13.95 16.06	**13.8**	2.0058 2.0078 2.0107	**2.0081**	**2.7**	2.0057 2.0075 2.0110	**2.0081**	**3.8**
	230	**29**	9.96 13.21 15.59	**12.9**	2.0053 2.0066 2.0101	**2.0073**	**2.9**	2.0053 2.0062 2.0097	**2.0071**	**5.5**
Monostarch phosphate	210	**28**	9.02 13.98 15.80	**12.9**	2.0052 2.0067 2.0099	**2.0059**	**2.7**	2.0047 2.0069 2.0101	**2.0072**	**5.2**
	230	**9**	8.65 15.76 16.44	**13.6**	2.0052 2.0045 2.0072	**2.0056**	**2.3**	2.0032 2.0053 2.0077	**2.0054**	**6.6**
Reference of distarch phosphate	210	**34**	11.00 13.90 16.64	**13.8**	2.0063 2.0071 2.0104	**2.0079**	**2.7**	2.0057 2.0076 2.0111	**2.0081**	**4.2**
	230	**29**	9.86 12.87 16.16	**13.0**	2.0050 2.0066 2.0098	**2.0071**	**3.1**	2.0046 2.0067 2.0097	**2.0070**	**4.7**
Distarch phosphate	210	**31**	10.06 13.71 15.38	**13.0**	2.0054 2.0070 2.0105	**2.0076**	**2.7**	2.0051 2.0074 2.0105	**2.0077**	**4.6**
	230	**19**	10.36 14.25 14.29	**13.0**	2.0067 2.0053 2.0084	**2.0068**	**4.5**	2.0051 2.0060 2.0089	**2.0067**	**5.1**

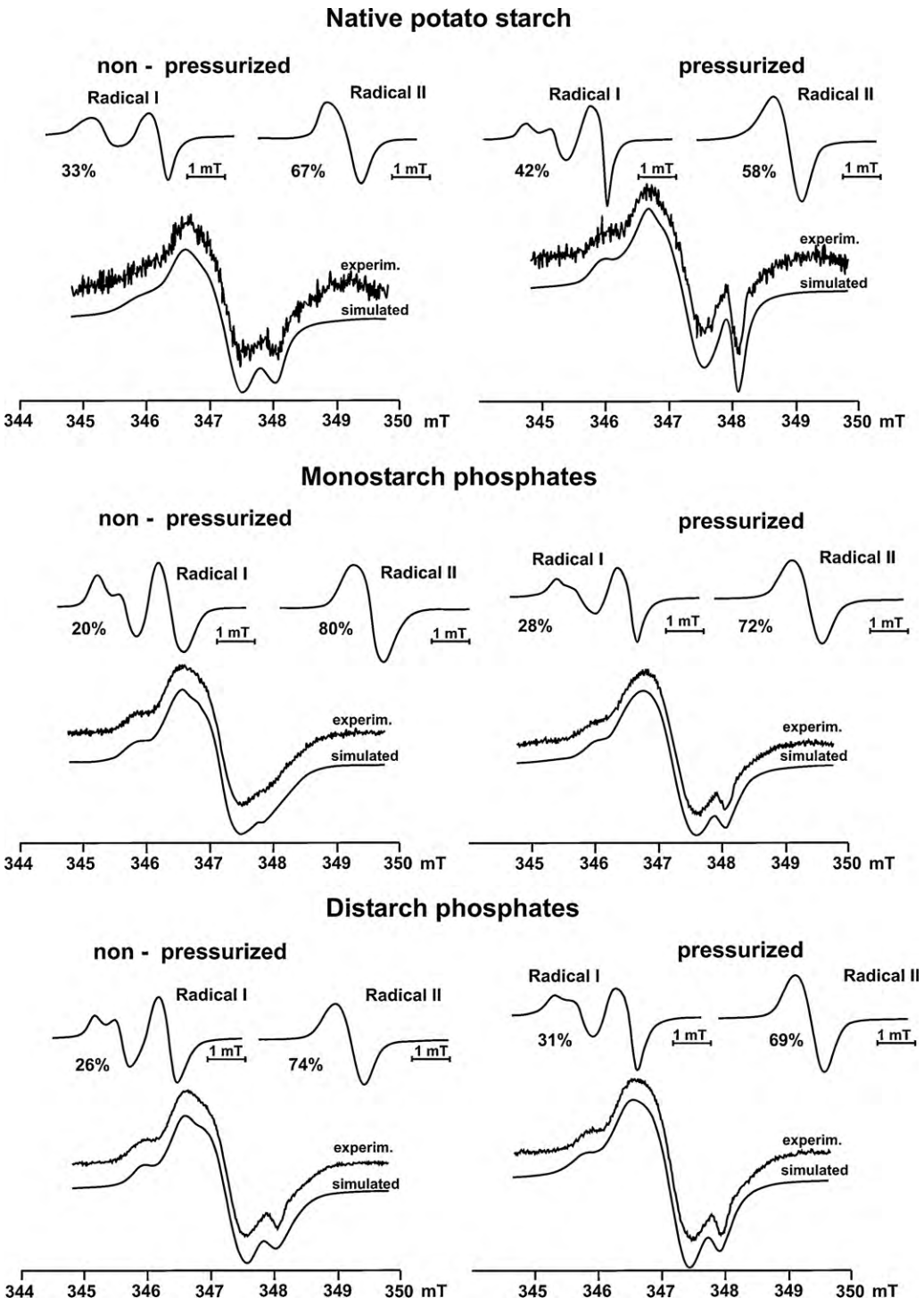

Fig. 5. EPR spectra of radicals generated at 210 °C in native and phosphorylated potato starch before and after teatment with high hydrostatic pressure.

Changes in the shape of the EPR signals of radicals occurring upon phosphorylation of the starch and its pressurization are presented in Fig. 5. The already discussed differences in the content of signal with HFS (radical I) are visible by comparing the non-phosphorylated samples with the phosphorylated ones (Fig. 6). Moreover, distinct sharpening of the EPR signals of radical I is observed in the pressurized samples (Fig. 5; Tables 2 and 3) which is consistent with previously published data [Błaszczak et al., 2008; Kruczala, Varghese, Bokria, & Schlick, 2003] proving that upon high pressure treatment crystallinity zones in starch decrease and the paramagnetic species became less restricted in their rotation.

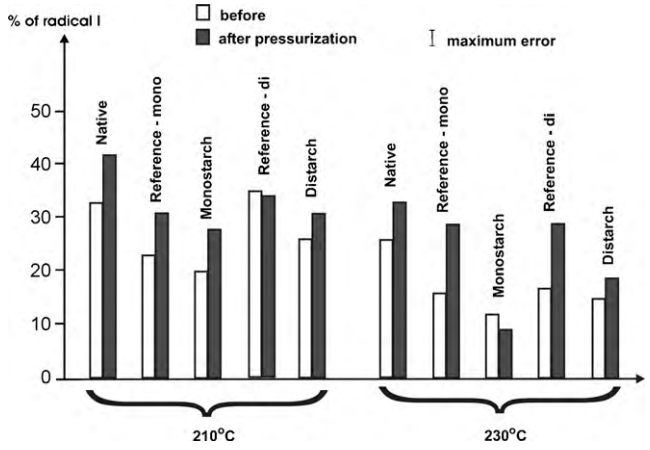

Fig. 6. Content of radical I generated thermally in phosphorylated potato starch before and after pressurization.

4. Conclusions

All the chemically and physically treated starches manifest alteration in thermodynamic parameters compared to native potato starch. Decrease in the melting enthalpy as well as in the degree of the crystallinity and increase in the gelatinization degree result directly from rupture of crystalline lamellae and weakening of starch structure. Changes in transition temperatures T_o and T_p provide indications on some cross-linking reactions occurring in the case of distarch phosphates. Treatment of the starch phosphates with high pressure affects formation of amorphous material that in excess of water forms homogenous and molecular dispersion.

The number of radicals generated thermally in phosphorylated potato starch is greater than in the native starch and in reference samples. This effect is more pronounced for monostarch than for distarch phosphates, most probably due to the cross-linking reactions occurring in the distarch phosphate samples.

Phosphorylation and pretreatment with high hydrostatic pressure have an opposite influence on the number of radicals generated in starch and on some of their EPR parameters. As a consequence the increase in the number of radicals caused by the presence of phosphorus may be reduced – especially in monostarch phosphates – by previous pressurization of the starch.

The shape of the EPR signal of radical I – being a component of the spectra of thermally generated radicals – is a good indicator of the starch pressurization. Decrease in crystallinity, occurring upon treatment of the starch with high hydrostatic pressure, makes the radical species less restricted in their motion, which results in sharpening of the EPR signals.

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