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ELECTRON PARAMAGNETIC RESONANCE STUDY OF THERMALLY GENERATED RADICALS IN NATIVE AND MODIFIED STARCHES

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Quantitative EPR spectroscopy was used for determination of the number of radicals generated thermally in native and modified starches and for characterizing the properties of these radical species. Native potato starch and maize starch with different amylose content modified by chemical (oxidation, phosphorylation) and physical (heating, high pressure, irradiation) methods were studied.

INTRODUCTION

Starch is, next to cellulose, the most abundant carbohydrate polymer accumulated by plants in the biosphere. It represents an important bio-renewable raw material for food industry. Starch has general formula $(C_6H_{10}O_5)_n$ and is composed of glucose units polimerized into amylopectin with highly branched chains and amylose with non-branched linear chains. Starches of different botanical origin contain 75-80 weight % of amylopectin and about 25 weight % of amylose, which are packed in the starch granule forming concentric amorphous and crystalline layers, resulting in ring structure of dimensions up to 100 μm in size.

In order to meet steadily increasing demands for improved specific starch functionalities, required by food industry, modifications of native starches are performed by chemical, physical and biological methods. Chemical modifications such as oxidation (Bidzińska, Dyrek, Fortuna, Łabanowska & Pietrzyk, 2004), esterification (Wurzburg, 1986), phosphorylation (Lim & Seib, 1992; Sang & Seib, 2006; Blennow, Houborg, Andersson, Bidzińska, Dyrek & Łabanowska, 2006)) lead to substitution of -OH groups in the starch matrix by other functional groups, e.g., –CHO, –COOH, $\equiv PO_4$, which results in changing of the chemical reactivity and rheological properties of the starch (Tomasik & Schilling, 2004). Physical modifications consisting on irradiation with γ- (Raffi & Agnel, 1983), UV- (Bertolini, Mestres, Colonna & Raffi, 2001), X- (Tomasik, Michalski, Bidzińska, Cebulska-Wasilewska, Dyrek, Fiedorowicz & Olko, 2008), γ - rays (Michalski, Bidzińska, Borowski, Dyrek, Olko, Stolarczyk, Swakoń, Tomasik & Wenda, 2010), or microwaves (Dyrek, Bidzińska, Łabanowska, Fortuna, Przetaczek & Pietrzyk, 2007), high hydrostatic pressure (Błaszczak, Bidzińska, Dyrek, Fornal & Wenda, 2008), mechanical (Kuzuya, Yamauchi & Kondo, 1999) or thermal treatment (Tomasik, Pałasiński & Wiejak, 1989; Tomasik & Jane, 1995) cause partial depolymerization of the starch and free radical formation. Biological modifications (Blennow, Bay-Smidt, Wischmann, Olsen & Møller, 1998; Blennow, Engelsen, Nielsen, Baunsgaard & Mikkelsen, 2002) consist on suppression or over-expression of appropriate enzymes of starch biosynthesis changing the size of the granules, chain length, content of incorporated trace elements etc. All these modifications might influence advantageously certain properties of the starch, important for food industry, e.g. stability during freeze-thaw cycles, gelatinization and pasting behavior, etc., but simultaneously some of them may enhance degradation of the starch through free radical formation (Tomasik *et al.,* 1989).

Literature data indicate that free radicals are not indifferent for human health, and may even cause serious diseases (Babbs, 1990; Steinberg, 1995) or accelerate cellular changes associated with aging (Ashok & Ali, 1999). Therefore determination of the factors controlling the free radical formation in experimental conditions commonly used for treatment of the food products containing starch is justified not only for scientific but also for social and economical reasons. Electron paramagnetic resonance (EPR) spectroscopy is an useful method for such investigations. The present work summarizes several applications of EPR technique as a tool for studying radical processes induced by heating of the starch. The obtained data lead to determination of the number and properties of radical species generated thermally in native and modified starches.

MATERIALS AND METHODS

Native potato and corn starches as well as waxy maize starch (amylopectin with trace amounts of amylose) were purchased from Sigma. Hylon VII containing 68 % of amylose, refined from high amylose maize, was produced by National Starch & Chemical, Food Starch, Poland. Modifications of the samples before EPR measurements by physical (thermal, high pressure, irradiation) and chemical methods (oxidation, phosphorylation, addition of sweeteners) are described in papers listed in References.

EPR measurements were performed 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 0.3 – 3.0 mW. The EPR spectra were recorded at room (20°C) and liquid nitrogen (-196 °C) temperatures. The number of spins was determined by comparison of the integral signal intensity of the investigated samples with that of the standard containing the known amount of paramagnetic centers. $VOSO_4.5H_2O$ diluted with diamagnetic K_2SO_4 (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 starch samples (native and modified) before and after thermal and/or high pressure 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 determinations of g factor values was equal to ± 0.001 and that of hyperfine splitting (HFS) constant A amounted ± 0.05 mT.

RESULTS AND DISCUSSION

Radicals generated in starches by conventional heating or by irradiation with microwaves

Heating of the potato or corn starch in the temperature range 200 – 250 °C, commonly used for preparing food, generates radicals with two-component anisotropic EPR signals (signal I and II) and g_{av} value equal to 2.006 - 2.007 (Fig.1) (Dyrek *et al.,* 2007). One of the components (signal I) exhibits HFS with A_{av} = 1.2 mT. The presence of two components was evidenced by measuring the EPR spectra at two microwave frequencies, *i.e.* in X and Q bands, and at different power levels. Signals I and II exhibit different saturation ability and at higher values of microwave power signal II became saturated whereas signal I, with HFS, is more distinctly visible.

The radical with signal I is generated thermally by abstraction of hydrogen ($H^\bullet$) from the glucose unit, most probably at C-1 atom (Fig.1). Signal I exhibits HFS due to the interaction of unpaired electron with nuclear spin of hydrogen (I = ½) localized at neighboring carbon atom C-2. Signal II, without HFS, represents carbon radical not interacting with vicinal hydrogen.

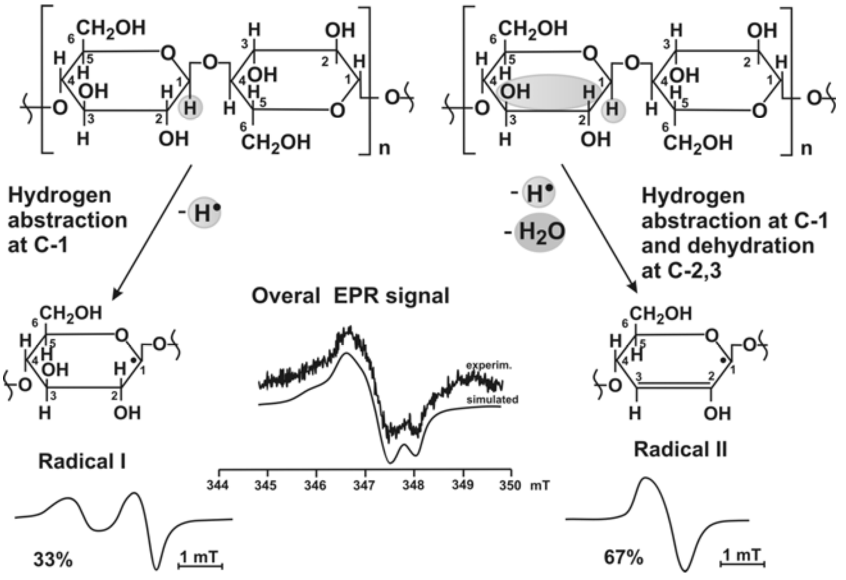

Fig. 1. Scheme of the thermal generation of radicals in potato starch at 210 °C.

The formation of this radical requires simultaneous abstraction of hydrogen at C-1 and dehydration, i.e. removal of H_2O molecule formed by hydrogen atom at C-2 and OH group at C-3. This process eliminates from the structure hydrogen atom responsible for appearance of HFS.

Similar radicals are generated by heating in the same temperature range the oxidized corn starch (Bidzińska *et al.,* 2004) or by irradiation with microwaves the native potato and corn starches (Dyrek *et al.,* 2007; Fortuna, Przetaczek, Dyrek, Bidzińska & Łabanowska, 2008). The relative content of radical I and radical II in total amount of radicals generated by thermal treatment of the native starches depends on their botanical origin and on heating temperature. For instance, after heating at 210°C the amount of radical I in native amaranth starch amounts about 10% whereas in native potato starch above 30%. With increasing temperature of thermal treatment the content of radical II in all starches increases at the expense of radical I. This effect is caused by the more intensive dehydration of the starches occurring at higher temperatures.

Cu^{2+} ions as a paramagnetic probe to study the mechanism of radical formation

Interaction of paramagnetic Cu^{2+} ions with the starch matrix leads to the formation of cooper complexes coordinating OH groups of the glucose units and water molecules (Łabanowska, Bidzińska, Dyrek, Fortuna, Pietrzyk, Rożnowski & Socha, 2008). The EPR parameters of these complexes give information about the changes occurring in the starch structure upon thermal treatment generating the radicals. The mechanism of this process consists on breaking of $R\text{-}CO\text{-}Cu^{2+}$ bonds and formation of transient $R\text{-}CO^{\bullet}$ species active in abstraction of hydrogen from the neighboring C atom of the glucose unit. As a result, the radical $RCO^{\bullet}$ is transformed into RCOH, whereas the unpaired electron became localized on the carbon atom from which the hydrogen was removed.

The Cu^{2+} probe was also used for investigation of the distribution of water molecules in the starch granules which underwent freezing and dehydration (Łabanowska, Bidzińska, Dyrek & Szymońska, 2006) and to study availability of phosphate groups in the granules of phosphorylated starches (Blennow *et al.,* 2006).

Radical processes in chemically modified starches

The number of radicals generated thermally in oxidized potato and corn starch is always higher than in similarly treated native starches (Bidzińska *et al.,* 2004). Evidently, transformation of OH groups of the glucose units into carbonyl or carboxyl groups destabilizes the starch structure. Modification of the starch by phosphorus with formation of monostarch or distarch phosphates improves some functional properties (gelatinization and pasting behaviour, stability during freeze-thaw cycles, *etc.*) but simultaneously changes the inclination of the starch to be thermally degraded (Błaszczak, Bidzińska, Dyrek, Fornal & Wenda, 2010a; Błaszczak, Bidzińska, Dyrek, Fornal, Michalec & Wenda, 2010b) with formation of the greater amount of radicals than in the native starch. Deterioration of the starch structure upon incorporation of phosphorus is more significant for monostarch than for distarch phosphates (Fig. 2), because in distarch derivatives some crosslinking occurs which stabilizes the structure.

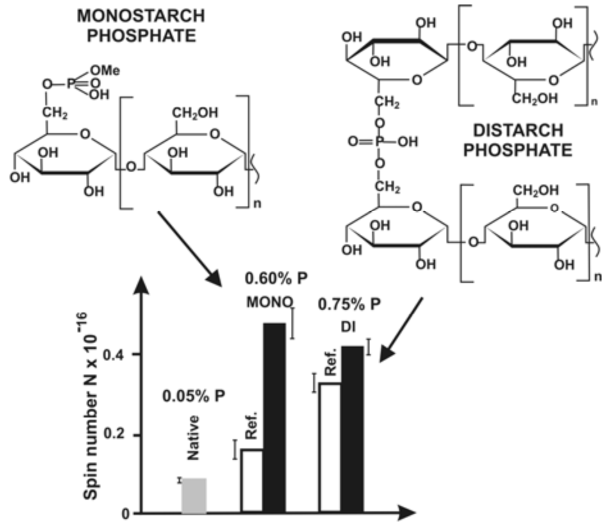

Fig. 2. Number of spins in radicals generated thermally at 210 °C in phosphorylated potato starch.

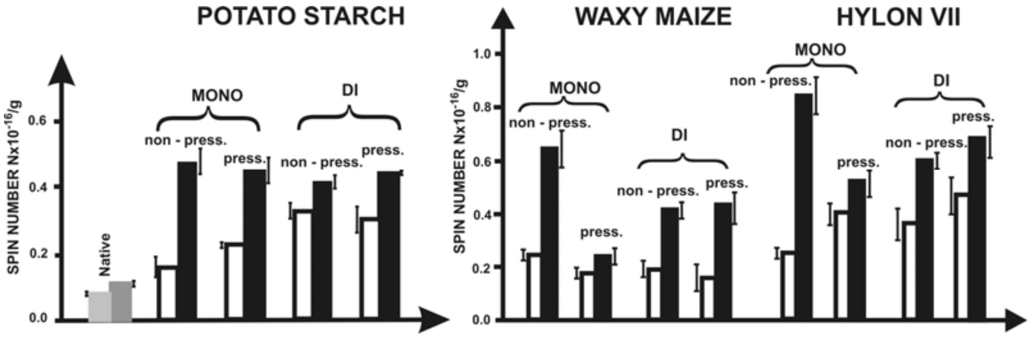

Fig. 3. Effect of pretreatment with high hydrostatic pressure on the number of radicals generated thermally at 210 °C in phosphorylated starches: potato starch, waxy maize and Hylon VII.

Artificial sweeteners, recommended for diet instead of sucrose in the case of diabetes change in a different way the behaviour of starch during thermal degradation: additives of acesulfam K lead to an increase whereas that of sorbitol to a decrease in the number of thermally generated radicals (Łabanowska, Dyrek, Bidzińska, Fortuna, Pietrzyk, Przetaczek & Rożnowski, 2008). The reasons of the opposite behaviour of these two sweeteners are different shape of their molecules and presence (in sorbitol) or absence (in acesulfam K) of the OH groups. Small molecule of sorbitol can easily penetrate the starch structure stabilizing it by using its own OH groups to form hydrogen bonds with glucose units. Acesulfam K does not exhibit any OH group and its heterocyclic ring with sticking out CH_3 group and three double bonded oxygen atoms may damage the elements of the starch structure during penetration. Such defects certainly facilitate formation of radicals.

Effect of pretreatment with high hydrostatic pressure on the number of thermally generated radicals

Free radicals were generated thermally in native and high pressure treated waxy maize starch, containing almost exclusively amylopectin, and in Hylon VII containing mostly amylose (Błaszczak *et al.,* 2008). Various stable radicals and short living radical species, stabilized by N-tert-butyl-α-phenylnitrone (PBN spin trap), were formed. It was found, that at given experimental conditions waxy maize reveals a higher tendency to generate radicals than Hylon VII. Pretreatment with high pressure resulted in diminishing of the amount of thermally generated stable and short living radicals in comparison to the non pressurized starches, the effect being again more pronounced for waxy maize than for Hylon VII.

Similar effect of the reduction in the number of thermally generated radicals was observed in phosphorylated starches after pretreatment with high hydrostatic pressure (Fig. 3) (Błaszczak *et al.,* 2010a,b).

Effect of irradiation with X- or γ-rays

Thermally treated potato and corn starches represent effective traps for radicals generated by ionizing X-ray or γ-radiation (Tomasik *et al.,* 2008; Michalski *et al.,* 2010). The most efficient scavengers of these new created radical species are radicals generated in starch by heating at 300 °C and above this temperature. In this temperature range radicals containing mostly component II of the EPR signal, not exhibiting HFS, are formed (see section "*Radicals generated in starches by conventional heating or by irradiation with microwaves"* of this paper). Annihilation of new radicals generated by ionizing radiation occurs with the rate dependent on the type of thermally generated radical scavengers and on the irradiation dose.

CONCLUSIONS

EPR spectroscopy appeared an effective method of detection and characterization of paramagnetic centers in starch, *e.g.* thermally generated radicals. Simulation of the spectra revealed their complexity and allowed identifying of particular component signals. An insight into the mechanism of radical processes induced by various treatments was acquired. The factors influencing reactivity, accessibility and stability of particular paramagnetic species were determined. The obtained data may be used for establishing suitable experimental conditions to control the number and features of radical centers generated in starch.

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