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INTERACTION OF STARCH POLYSACCHARIDES AND THEIR MIXTURE WITH WATER MOLECULES AND MODEL LIPIDS. ESR STUDY

Summary

Electron spin resonance (ESR) was used in order to study interaction of starch polysaccharides (amylose and amylopectin), their mixture and gelatinized potato starch with water molecules and lipids upon cooling. Different spin probes were used, on the one hand spin-labelled stearic acid (5-DSA), which limited lipids, and on the other hand the water soluble probe 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempol), which was sensitive to changes in dynamic water phase associated with the temperature-induced polysaccharide gel formation. It was shown that interaction between gelatinized starches and lipids related to mainly on presence of amylose macromolecules in the system. On the other hand, interaction between amylopectin macromolecules and lipids takes place also.

Introduction

Depending on starch origin and functional properties gelatinized starches are widely used in complex food systems such as meat products (for example, ham-type products, hamburgers), emulsion sausages, for example, Frankfurter and Bologna types of sausages, and other [1, 2]. Important features of such products, besides sensory attributes (consistency, taste, appearance and juiciness) are water holding and fat holding therefore gelatinized starches are used as gelling or thickening agents. When the treatment leading to the final products implies distruption of natural components, either tissue or cell level, the systems, once sufficiently hydrated at suitable temperatures, display heterogeneity related to phase separations which are mainly driven by the thermodynamic incompatibility between the different polymers components, like

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polysaccharides versus proteins [3], or even between different polysaccharides like amylose versus amylopectin [4]. This implies water partition into different phases and different kinds of interactions between water molecules and substrates. Additionally, starch polysaccharides may interact with minor components of complex food systems such as vegetable and animal fats. However, some problems concerning interaction gelatinized starches and starch polysaccharides, in particular, with water, lipids and fatty acids molecules remain not quite discussed till now.

Some methods, such as calorimetry and tradition thermal analyses, that allow to detect the macroscopic properties, like heat capacity and thermodynamic activity, can be sensitive only for the changes occurring in the phases where water has the highest mobility. Others, like Nuclear Magnetic Resonance (NMR) and Electron Spin Resonance (ESR), on the contrary can provide information about many coexisting states of water molecules, which are separated from one another because of the different relaxation times related to the short-range mobility. In particular, as was shown by ESR study the rotational diffusion coefficient (D_{rot}) decreased monotonically with decreasing temperature in the system of gelatinized potato starch-water [5]. Investigating of starch gels, Baster and Lechert [6] established that the coefficient of self-diffusion of water molecules is roughly proportional to the square of the water fraction. At present it is known that spin-lattice relaxation times ($1/T_2$) measured as a function of water contents indicate a wide distribution of correlation times in the processed starch-water systems [7]. As has been shown earlier complex relaxation of water molecules is observed due to formation of amylose aggregates at starch concentrations close to critical gelation concentration or close to critical gelation temperature [8, 9]. Lifetime of these aggregates was comparable or exceeded the relaxation time of water molecules, which formed during gelatinization of starch and dissolution of maltodextrin. However up to now it is not quite understood what of polysaccharides (amylose or amylopectin) is determined the mobility of water molecules in starch-water systems. Additionally, it is not quite clear whether mobility of water molecules in real starch systems can be described using additive scheme assessing of mobility of water molecules and content of starch polysaccharides in the simple polysaccharides systems (amylose – water, amylopectin – water).

In contrast to investigation devoted to interaction of native starches with lipids model (spin probes such as spin-labelled stearic acids), the data concerning interaction in systems of gelatinized starch – spin probe, amylose (amylopectin) – spin probe are not enough. It is known that upon cooling of the potato (maize) starch – water systems the sharp decrease of mobility of spin probe was observed close to the critical gelation temperature [5, 10]. Additionally, it is known that amylose macromolecules can form inclusion complexes with low molecular substances such as fatty acids, lipids and aroma compounds [11, 12]. It is suggested that side chains of amylopectin macromole-

cules can form inclusion complexes also however directly evidence of existing such complexes we didn't find at the analysis of the published data [13, 14]. For better undestanding of features of interactions of the gelatinised starches with water, fatty acids and lipids in complex food systems we study interactions amylose, amylopectin, their mixture and potato starch with these low molecular substances by ESR. Spin-labelled stearic acid (5-DSA) was chosen as lipid model. A water-soluble stable radical (Tempol) was also used in order to probe changes in the properties of aqueous continuous phase.

Materials and methods

Potato starch (20–25% amylose according to literature data [15]) was obtained from Paille (France). The content of proteins and lipids in commercial potato starch was very low and constitutes 0.06% w/w (proteins) and 0.05%w/w (lipids) on dry substance [16]. Amylose (EC N 232-685-9) and amylopectin (EC N 232-911-6) from potato were purchased from Sigma, USA.

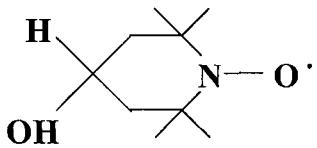
Paramagnetic homologue of stearic acid (5-Doxyl stearic acid (5-DSA)) used as lipid model and Tempol (4-hydroxy-2,2,6,6-tetra-methylpiperidine-1-oxyl) were obtained from Sigma (USA).

The chemical structures of these spin probes are shown on Fig. 1. 40% (w/w) dispersions of amylose, amylopectin, their mixture and potato starch were studied. Spin-labelled stearic acids were poorly soluble in water and were introduced to water as acetone solutions (as described in [17, 18]) The acetone concentration in water did not exceed 1 w/w %. The system was sonicated and mixed intensively for 1 hour at 50–55°C to evaporate the acetone. The concentration of the spin probe in water was $2.6 \cdot 10^{-4}$ M. Potato starch with moisture contents of 14.9% (w/w) was used for preparation of dispersions. Aqueous solutions of spin-probe (0.53 g) were added to investigated polymer systems (0.47 g) for preparation of dispersions (40% w/w) at room temperature. As Tempol was more soluble in water, an aqueous Tempol solution was prepared and added to the starch dispersion in order to reach a 3.6×10^{-4} M concentration of Tempol in the investigated dispersions.

After 24 hours incubation, the samples of starch preparations with different water contents were placed into glass tubes ($d \approx 1$ mm), which were sealed to prevent dehydration during heating. For preparation of a macromolecule dispersion of investigated systems the samples were heated up to 115°C [19]. ESR spectra were recorded with a controlled temperature EPR spectrometer (Radiopan, Poland), using cooling cycles over a temperature range of 115–25°C by stepwise fashion at interval 10°C. Samples were allowed to come to thermal equilibrium for three minutes at each temperature before spectra recording. The microwave power was below saturation. ESR spectra

were recorded three times per sample. No differences were observed between spectra recorded at the same temperature at a given starch content.

a) Tempol



b) 5- Doxyl stearic acid (model of lipid)

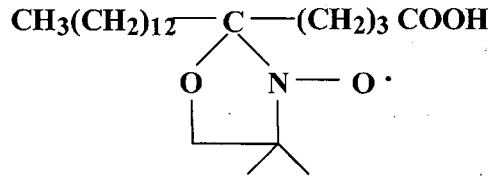


Fig. 1. Chemical structures of spin probes Tempol (a) and 5-doxyl stearic acid (model lipid) (b).

For the description of the spin probe mobility, the rotational correlation time (τ_c) and the rotational diffusion coefficient (D_{rot}) were usually used [20]. The rotational correlation time was determined from features of the spectra obtained with fast isotropic rotation according to the Freed and Fraenkel theory [18, 20].

The following equation was used:

$$\tau_c = 6.65\Delta H_{(+1)}(\sqrt{I_{(+1)}/I_{(-1)}} - 1)10^{-10}, \text{ sec} \quad (1)$$

where: $\Delta H_{(+1)}$ is the peak-to-peak width of the low field line (Gs);

$I_{(+1)}$ and $I_{(-1)}$ are the heights of the low and high field lines, respectively.

The rotational diffusion coefficient was calculated from the following relation:

$$D_{rot} = 1/(6\tau_c), \text{ sec}^{-1} \quad (2)$$

The program of Freed [20] modified by V. Timofeev [21, 22] was used for the simulation of ESR spectra in the region of slow motions. The calculations were made according to the model of isotropic rotation, and the following values for the electron-spin parameters of radicals in the presence of amylopectin were used:

$g_{xx} = 2.088$; $g_{yy} = 2.061$; $g_{zz} = 2.0027$; $A_{xx} = 6.3$ Gs; $A_{yy} = 5.8$ Gs; $A_{zz} = 33.6$ Gs [23], where g_{xx}, g_{yy}, g_{zz} – are the main components of Zeeman interaction tensor (g-tensor), and A_{xx}, A_{yy}, A_{zz} are the main components of hyperfine interaction tensor. A better agreement between experimental and calculated ESR spectra in the presence of amylose was observed when used the following values for electron-spin parameters of radicals:

$g_{xx} = 2.088$; $g_{yy} = 2.061$; $g_{zz} = 2.0027$; $A_{xx} = 6.5$ Gs; $A_{yy} = 6.0$ Gs; $A_{zz} = 33.8$ Gs.

Calorimetric investigations of amylose and amylopectin dispersions in 5-DSA aqueous solution were performed using a high sensitivity differential scanning microcalorimeter DASM-4 (Moscow, Russia) from 10–130°C with a heating rate of 2 Kmin^{-1} and pressure of 2.5 bar. Deionised water was used as a reference material. Calorimetric investigations were made upon different molar ratio [radical]/[polymer]. For deter-

mination molar ratio of radical/polymer there were used the values of molecular weights from [16]. The molar ratio radical/polymer was 1:25 for system 5DSA-amylose, for system 5DSA – amylopectin there were used the following molar ratio radical/polymer: 6290:1, 50649:1. Measurements were obtained both for dispersions prepared at the same day, and after 6 days storage (for one dispersion of 5DSA-amylopectin at molar ratio 50649:1).

Results and discussion

Mobility of the water-soluble spin probe

The ESP spectra of Tempol in water and 40% aqueous (w/w) molecular systems of amylose, amylopectin, their mixture as well as gelatinized potato starches at 25°C after cooling are shown in Fig. 2. Symmetrical triplet signals characteristic of spin probes with a "fast rotation" were obtained for all investigated samples, it'd be like to mark that the same ESR spectra of Tempol were observed in the presence of native maize and potato starches [5, 10, 12].

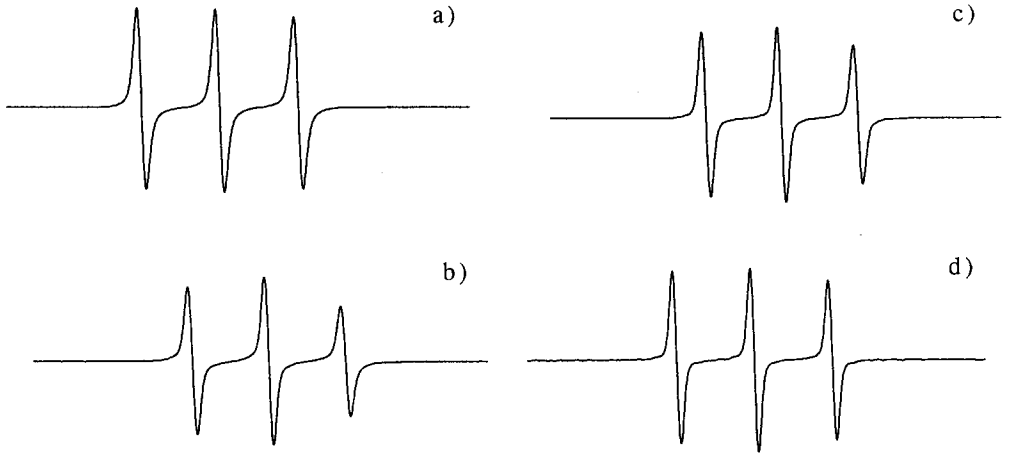


Fig. 2. ESR spectra of Tempol in water (a), in the presence of 40% (w/w) dispersions of amylose (b), amylopectin (c) and potato starch (d) at 25°C after cooling.

Tempol was chosen, since behaviour of this radical simulated the behaviour of water molecules in the system. Only one probe population was observed, suggesting a homogeneous distribution of probes in water phase of the molecular dispersions. This also demonstrates the absence of any direct interaction between the spin probe and starch polysaccharides, i.e. the presented spectra characterize the mobility of water molecules in the dispersion. It should like to note that D_{rot} values for Tempol in bio-

polymer systems were found to be smaller than the values for the probe in bulk water at the same temperature. The differences in D_{rot} could be attributed to water microviscosity in polymer systems.

The dependences of the rotational diffusion coefficient (D_{rot}) on temperature for all investigated systems upon their cooling are shown in Fig. 3. As shown in Fig. 3, the mobility of spin probe Tempol in amylopectin-water system is higher than in amylose-water system. It is known, that upon cooling the amylose macromolecules, in contrast with amylopectin macromolecules, form aggregates followed by its organized three dimensional gel network [8, 9]. That is the reason of the lower spin probe mobility in the amylose-water system as compared with amylopectin-water system.

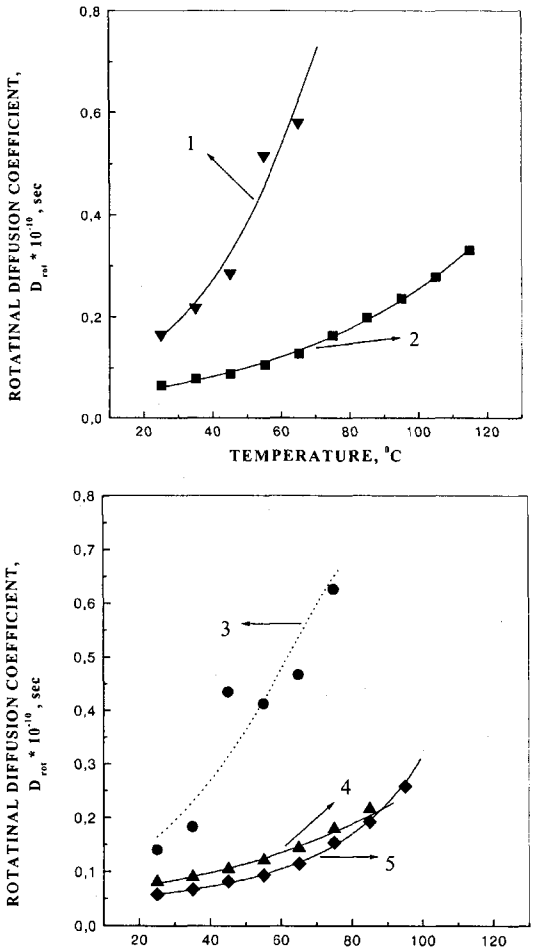


Fig. 3. Dependences of rotational diffusion coeffient on temperature for 40% (w/w) dispersions of amylopectin (1), amylose (2), theoretical (3) and experimental (4) obtained dependence for mixture of amylose and amylopectin (25 w/w % amylose and 75% (w/w) amylopectin), potato starch (5).

Since amylose and amylopectin macromolecules were incompatible in aqueous medium [4] it could be suggested that the observed changes of D_{rot} for model systems (amylose-amylopectin-water) (Fig. 3), can be described by means of additive scheme (3):

$$D_{rot} = D_{rot\ aml}\ \alpha_{aml} + D_{rot\ alp}\ \alpha_{amp} \quad (3)$$

where $D_{rot\ aml}$ and $D_{rot\ alp}$ are the rotational diffusion coefficient for the systems of amylose-water and amylopectin-water, respectively, α_{aml} and α_{amp} were amylose and amylopectin content (%) in model system. To check this assumption, the rotation mobility of so-called "model" system and real potato starch were investigated. "Model" system was realised mixture of amylose and amylopectin, at that weight content of each biopolymer in the mixture was the same as in real potato starch, i.e. 25% (w/w) amylose and 75% (w/w) amylopectin. The comparison of the data obtained shows (Fig. 3b) that generally the experimental and calculated values for model systems differed from one another. So the rotational mobility of system could not be described using additive scheme. The differences between experimental and calculated data for model system could be due to next reasons: (i) the lack of assessment of parameter characterizing mobility of water molecules in interface, since amylose-amylopectin-water is incompatible system [4]; (ii) formation of aggregates and formation of three dimensional gel network upon cooling [8, 9].

At the same time the rotational mobility of spin probe Tempol, which was simulated the behaviour of water molecules in investigated systems, was practically the same in real gelatinized potato starch-water system and in "model" amylose-amylopectin-water system. Moreover the spin probe mobility in the "model' system and in real gelatinizated potato starch-water system was close to spin probe mobility in amylose-water system. From these results it was possible to conclude that the water mobility in real gelatinizated potato starch-water system was mainly related to the presence of amylose macromolecules

Mobility of the spin - labelled stearic acid (5-DSA)

5-DSA was a spin labelled fatty acid with the nitroxide moiety close to the lipid polar head (Fig. 1b). The spectra of 5-DSA in water at 25°C and evolution of ESR spectra in the presence of amylose and in the presence of amylopectin during cooling are shown in Fig. 4. Similar spectra were observed for 40%, 50% and 60% aqueous dispersions of gelatinized potato starch [5]. The ESR spectra of 5DSA in water corresponded to a spin probe with a "fast rotation" (rotation correlation time $\tau_c = 3 \cdot 10^{-10}$ sec; $D_{rot} = 5.5 \cdot 108\ sec^{-1}$) (Fig. 4(a)). After addition of amylose, amylopectin and gelatinized potato starch to the 5-DSA aqueous solution, the motional behaviour of the spin labelled fatty acid drastically decreased. Spectra became characteristic of low-mobility

radicals (i.e. powder-like spectrum). The dominant feature of spectra was the broadened anisotropic line pattern (Fig. 4) indicating greatly slowed down motions as compared with spectra of 5-DSA in water. It could be concluded that interactions between 5-DSA and starch polysaccharides or gelatinized potato starch took place. It was necessary to note that if interaction of amylose macromolecules with 5-DSA could be expected [10, 11], the fact of the interaction of amylopectin macromolecules with 5-DSA, which was considered as model for starch lipids [5, 12],was observed first of all.

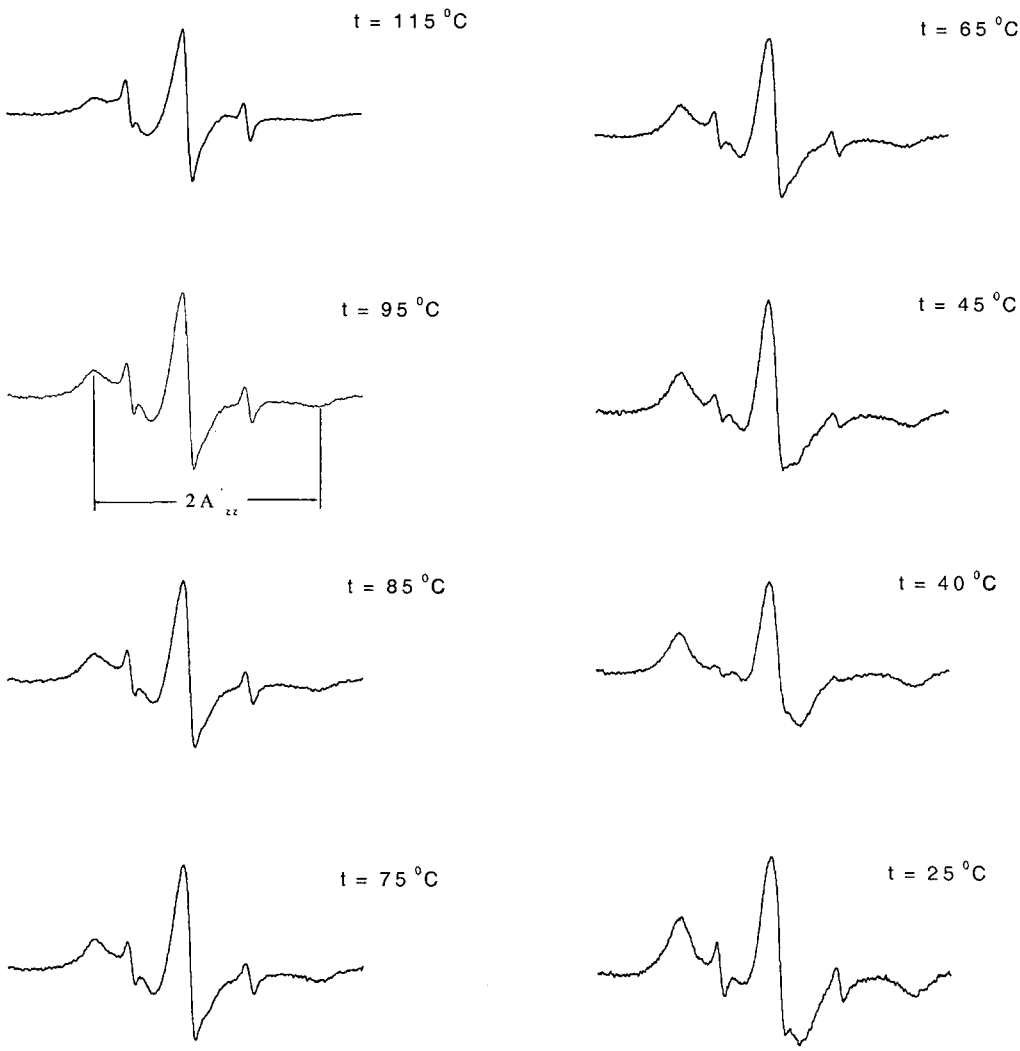


Fig. 4a. ESR spectra of 5-DSA in water at 25°C and evolution ESR spectra of 5-DSA upon cooling in the presence of 40% (w/w) dispersions of amylose.

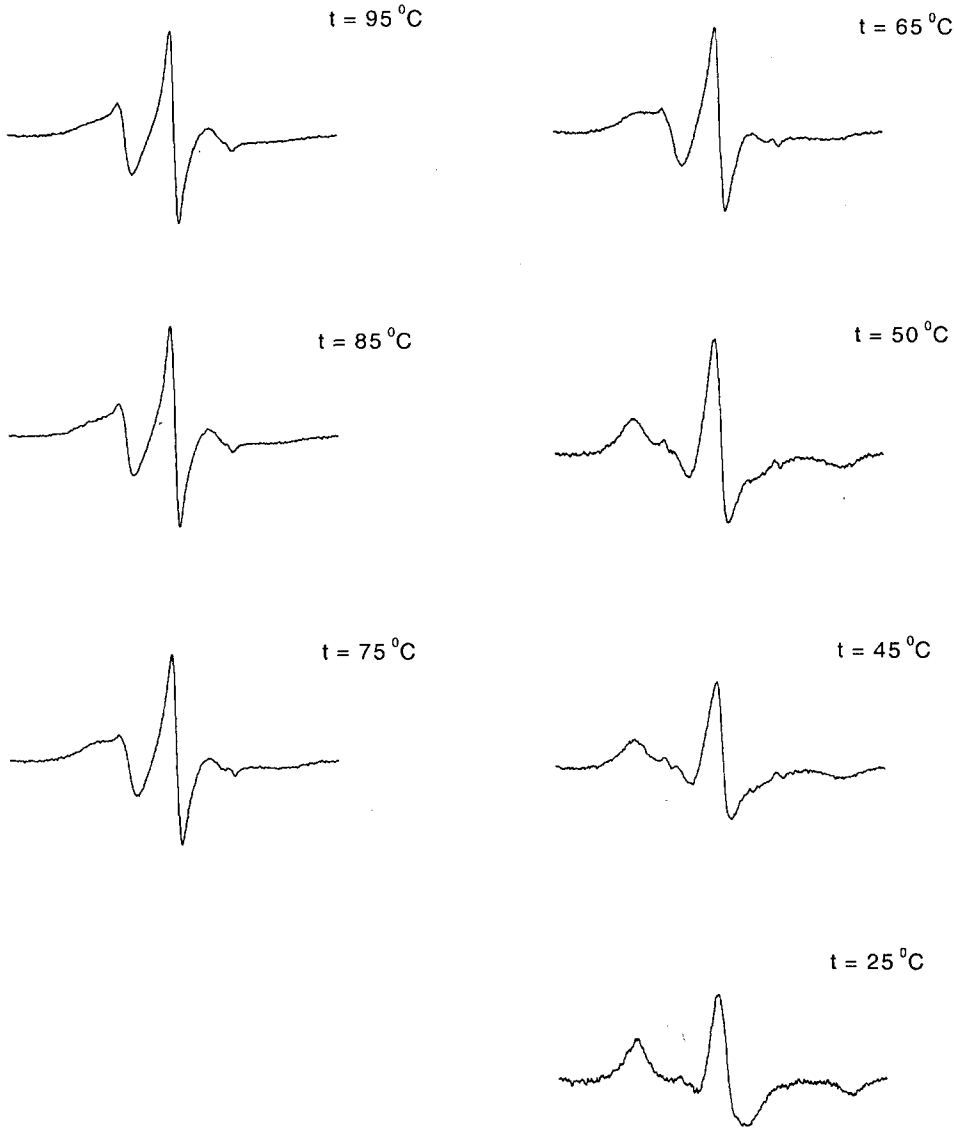


Fig. 4b. ESR spectra of 5-DSA in water at 25°C and evolution ESR spectra of 5-DSA upon cooling in the presence of 40% (w/w) dispersions of amylopectin.

Upon cooling, the powder-like spectra appeared indicating that the mobility of the probe decreased. This meant that more immobilised spectra of 5-DSA were observed. In investigated temperature range two components with "fast" (narrow line spectrum) and "slow" (broad line spectrum, see, for example, Fig. 4(b) at 95°C) motions respectively were superimposed. Fig. 5 presents the evolution of hyperfine extreme separa-

tion ($2A'_{zz}$) as a function of temperature for 5-DSA for all studied systems. The splitting (the value of extrema separation) was higher, the mobility of radical was smaller in the system. Taking into consideration that value of extrema separation was higher in the presence of amylose than in the presence of amylopectin it could be supposed that the interaction between lipids and amylose was stronger than that in the case of amylopectin.

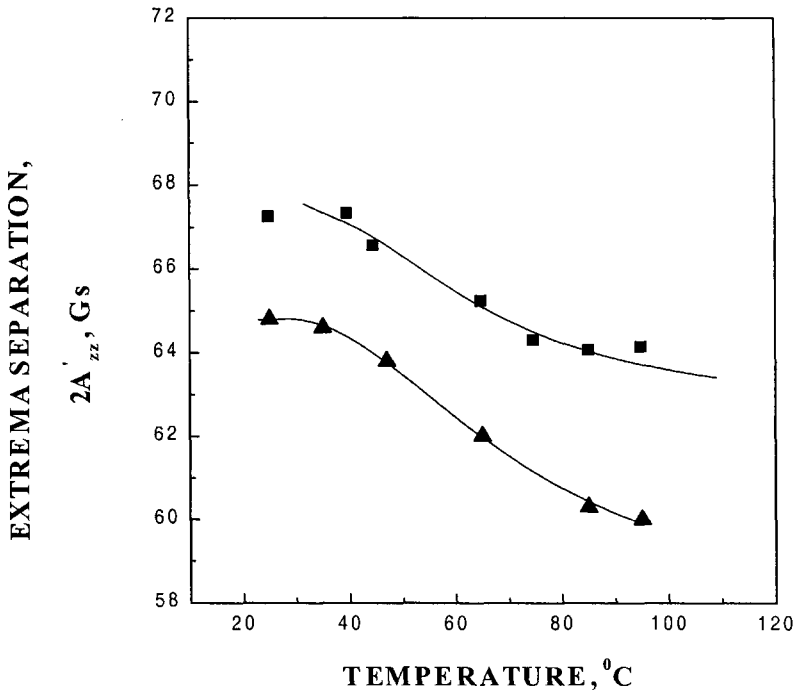


Fig. 5. Dependence extrema separartion for 5-DSA on temperature in the presence of 40% (w/w) dispersions of amylose (■) and amylopectin (▲).

Calculations were carried out to simulate spectra of 5-DSA, in the presence of 40% amylose and amylopectin at 95°C, when apparently, two populations of spin labelled lipids with different mobilities coexisted: "fast" rotating radicals and radicals with slow motions. For both populations isotropic rotation was assumed. The results of this calculation are shown in Fig. 6. Although our model was relatively rough, a relatively good correlation between calculated and experimental spectra was obtained. The simultaneous presence of two different populations suggested a heterogeneous distribution of the labelled fatty acids in different environments. These two populations could be associated to relativelly "free" spin labelled lipids for the most mobile population, whereas the "slow" rotating radicals could be associated to spin-labelled lipids forming of inclusion complexes.

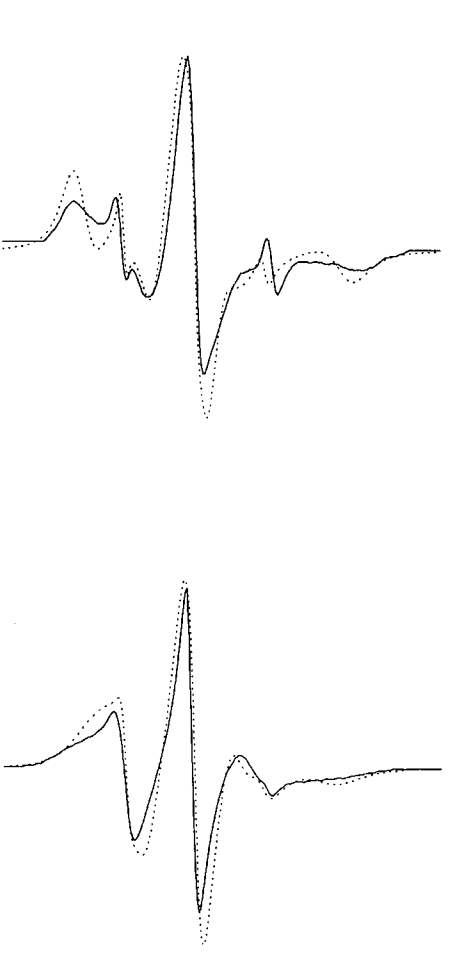


Fig. 6. Experimental (——) and theoretical (------)ESR spectra of 5-DSA in the presence of 40% (w/w) dispersions of amylose (a) (theoretical spectrum was calculated as superposion of spectra with correlation times $\tau_1 = 1.5\ 10^{-8}$ sec (molar part is 98%) and $\tau_2 = 5\ 10^{-10}$ sec (molar part is 2%)) and amylopectin (b) ($\tau_1 = 5\ 10^{-9}$ sec (molar part is 93%) and $\tau_2 = 1.2\ 10^{-9}$ sec (molar part is 7%) at 95°C.

In order to confirm this assumption 5DSA-amylose and 5DSA-amylopectin systems were studied by DSC technique. DSC-thermograms of investigated systems are shown in Fig. 7. The phase transition of first kind for system of 5DSA-amylose at the temperature 90°C was observed (Fig. 7(a)) inspite of that molar raio 5DSA/amylose was lower in DSC measurements as compared with ESR measurements. It is well known that the melting of amylose-lipid inclusion complexes takes place at this temperature. Therefore, populations of radicals associated with "slow" rotating radicals in

the presence of amylose could be associated to spin-labelled lipids forming of inclusion complexes.

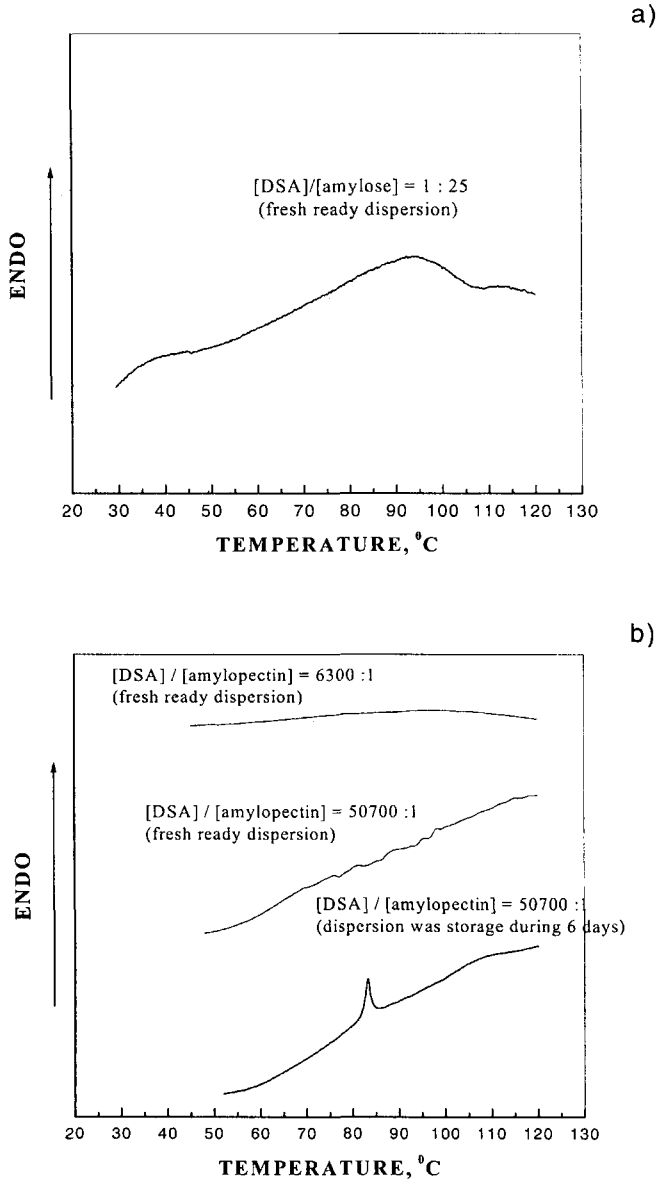


Fig. 7. DSC traces for amylose dispersion in aqueous solution of 5DSA (a) and for amylopectin dispersions (b).

In contrast to 5DSA-amylose system, the phase transitions for 5DSA-amylopectin system with different molar ratio radical/ amylopectin even upon higher than one in

ESR measurements could not be observed on DSC-traces. This meant that the formation of inclusion complexes in the 5DSA-amylopectin system was not observed. Hence, population of radicals with "slow" rotating in the presence of amylopectin was characterized probably formation of complexes other nature. Apparently such complexes were stabilized by adsorption interactions. Therefore the value of correlation time of 5DSA characterizing "slow" radicals in the presence of amylopectin was less than one in the presence of amylose. This meant that interaction amylose with lipids was stronger than amylopectin with lipids. This fact was confirmed our supposition which was discussed above. Although the phase transition of first kind on DSC-traces for 5DSA-amylopectin system after 6 days of storage at 85°C is observed, i.e. was most likely that the formation of amylopectin-inclusion complexes is kinetic process.

Conclusion

Our investigations show that in real three components systems (gelatinized starch-water) interactions the starch polysaccharides macromolecules with water molecules and lipids were related to amylose macromolecules. The interaction of amylose macromolecules with water was caused by formation of aggregates and three dimensional gel network upon cooling. The formation of lipid inclusion complexes was caused by main contribution of amylose macromolecules. On other hand the interaction between amylopectin and lipids also took place.

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ODDZIAŁYWANIE MIĘDZY POLISACHARYDAMI SKROBIOWYMI I CZĄSTECZKAMI WODY ORAZ MODELOWYMI LIPIDAMI. BADANIA ESR

Streszczenie

Za pomocą elektronowego rezonansu spinowego (ESR) zbadano, powstające po chłodzeniu, oddziaływania między polisacharydami skrobiowymi (amylozą i amylopektyną), skrobią i zżelowaną skrobią ziemniaczaną a wodą i lipidami. Zastosowano różne wskaźniki spinowe pozwalające badać zmiany w dynamice fazy wodnej związane z powstawaniem indukowanych temperaturą żeli. Pokazano, że w oddziaływaniach między zżelowaną skrobią i lipidami uczestniczy głównie amyloza chociaż obserwuje się też oddziaływania między lipidami i amylopektyną.