Binding of the Trace Elements: Cu(II) and Fe(III) to the Native and Modified Nutritive Potato Starches Studied by EPR

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The Cu(II) and Fe(III) ions have been adsorbed by four potato starches of different degrees of oxidation (different numbers of COOH groups replacing host CH_2OH groups): native (no oxidized), white (pudding) with oxidation degree of 0.04%, gelating (0.1%), and LUBOX starch (0.5%). Concentration of the ions in starches was determined from atomic absorption and EPR spectrum intensity. For small concentration of the adsorbed ions (below 4 mg/g) nearly all ions are adsorbed from the solution. EPR shows that adsorbed copper(II) ions are chemically bonded to the starch molecules (preferably) at COOH sites and uniformly dispersed in the starch structure. The complexes are typical of octahedral or square-quadratic coordination with spin-Hamiltonian parameters $g_{\parallel} = 2.373$, $g_{\perp} = 2.080$, $A_{\parallel} = 12.1$ mT, $A_{\perp} = 1.0$ mT. For higher concentrations the Cu(II) displays a tendency to clustering. Iron(III) ions are introduced into starch in a form of clusters mainly, even for the smallest concentration. The highest concentrations of both Cu(II) and Fe(III) were observed in LUBOX starch having the highest degree of oxidation.

PACS numbers: 76.30.–v, 87.15.–v

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1. Introduction

Starches from different sources are commonly used in food industry. They offer a range of functional properties especially after their physical and chemical modifications. It is known that starches can adsorb various metals such as alkali metals and transition metals [1 and references therein]. This gives a possibility to introduce a controlled amount of biometals into foods containing starch to supply the daily diet. In the USA the recommended daily amounts (RDA) for Cu and Fe are 0.9 mg and 8–18 mg, respectively, whereas in Poland — 2 mg and 15–18 mg, respectively. Iron is the biometal that is necessary for producing the red blood cells and for redox processes. Its deficiency is considered to be the commonest worldwide nutritional deficiency and affects approximately about 20% of the world population. Lack of iron may lead to unusual tiredness, shortness of breath, a decrease in physical performance, and learning problems of children and adults, and may increase a chance of contracting an infections [2]. Iron deficiency anemia is a major nutritional problem in the developing word and affects all segments of the population [3]. It has been known for a long time that there exists a relationship between iron and copper. The ceruloplasmin, the main cuproprotein in plasma which is produced in liver, is essential for a mobilization of iron from storage sites such as liver ferritin. This process requires a change in valence state of iron, the Fe(II) has to be oxidized by ceruloplasmin because only Fe(III) is incorporated into transferrin [4]. Copper deficiency rapidly lowered the level of ceruloplasmin in plasma and as a result of that accumulation of iron in liver and some other organs is observed [5, 6]. Moreover, copper is an essential trace element that serves as a cofactor for several enzymes and proteins crucial for physiological and biological functions [7, 8].

Starch $(C_6H_{10}O_5)_n$ (n = 50–thousands) is a polysaccharide polymer composed of repeating glucose units. Starch molecule consists of two fractions: amylose and amylopectin. Amylose (α(1-4) glucose) is a very long molecule composed of 200–200000 glucose subunits linked by α(1-4) bond forming unbranched helix with extensive hydrogen bonding. Amylose constitutes of about 20–30% of an ordinary starch. Amylopectin is branched via α(1-6) bonds polysaccharide fraction of starch. Branch points exist in every 20–30 residues. Each amylopectin molecule contains up to two million glucose residues. The starch components are shown in Fig. 1. Amylose helices and branched amylopectins are associated through hydrogen bonds forming concentric amorphous or crystalline layers resulting in a globular shape of the starch grains of the size of $1-100$ μm.

Starch is often modified by physical, chemical, and enzymatic treatments in order to improve its functional properties. One of the chemical modification is oxidization. Oxidized starch is produced by the reaction with a specified oxidizing agent under controlled temperature and pH [9, 10]. Starch for the food industry is usually oxidized by the sodium hypochlorite. Hydroxyl groups on starch molecules are first oxidized to carbonyl groups and then to carboxyl groups [10]. Therefore,
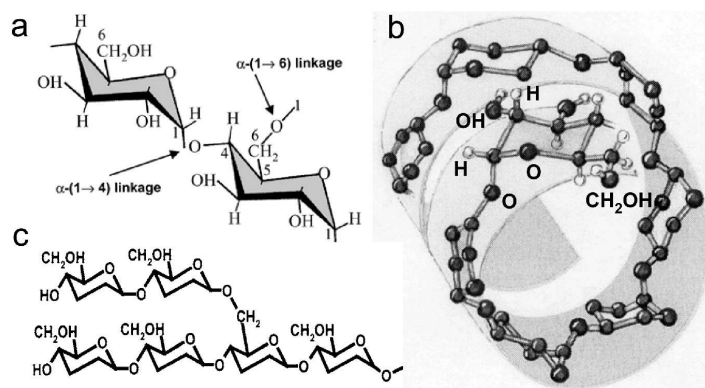
Fig. 1. Starch molecular structure: (a) amylose chain formed from 1-4 linked glucose molecules with marked 1-6 side linkage for forming an amylopectin branched structure, (b) α-helix of amylose, (c) amylopectine branched structure.

the number of the carboxyl and carbonyl groups indicates the level of the oxidation. A unique molecular structure of native and modified starches gives a possibility to introduce a controlled amount of biometals into foods containing starch to supply the daily diet. Even distribution of individual metal ions within carrier material is crucial in proper fortification, therefore conditions of biometal bonding to starch molecules, localization and electronic structure of metal complexes in starch granules, and conditions for their release are currently the main fundamental problems.

In this paper we present results of EPR and optical absorption studies of copper(II) and iron(III) ions introduced into the potato starch and its modifications with different degrees of oxidation.

2. Experimental

The four commercial starches of potato origin produced by WPPZ Luboń (Poland) were studied. There were three oxidized starches of different degrees of substitution: "Pudding powder" (white starch) (E1403), "Gel-forming food starch" (gelating starch) (E1404), and "LUBOX" (E 1404) containing 0.04%, 0.1%, and 0.5% carboxyl groups, respectively. Native potato starch "Superior Standard" was used as reference material.

The metal adsorption capacity of the experimental starches was determined by the bath method. To this end metal salts were adsorbed by granular starch samples from aqueous solutions containing different amounts of $CuSO_4 \cdot 5H_2O$ or $Fe_2(SO_4)_3 \cdot nH_2O$. Copper(II) sulfate (analytical grade, Fluka Chemie AGI) or iron(III) sulfate (exsiccated and extra pure, Riedel-de Haën) were dissolved in deionized water. 2, 4, 6, 8, or 10 ml of resulted solutions contained 1 mg of metal

in 1 cm^3 of solution were mixed with 2 g of starch sample. Deionized water was added to make a suspension containing 20 ml aqueous phase, then the mixtures were shaken at 50°C for 1 hour. After completion of the adsorption process the solutions were centrifuged, starch samples were three times washed out with water and dried. The metal salts contents in starch samples were determined with Varian AAS atomic absorption spectrometer after wet mineralization with HNO$_3$ acid.

Electron paramagnetic resonance (EPR) spectra were recorded at room temperature on a Radiopan SE/X-2547 spectrometer at about 8.96 GHz with a cylindrical TE$_{011}$ cavity and 100 kHz magnetic modulation. Concentration of Cu(II) and Fe(III) ions was small enough to allow observation of EPR spectra of individual complexes, whereas in paper [1] the concentration was much higher and non resolved (non informative) EPR spectra were recorded, or even signals from inclusions of a paramagnetic salt (like CuSO$_4 \cdot$ 5H$_2$O) were observed.

3. Results and discussion

Atomic absorption (AA) results and EPR spectra show that Cu(II) and Fe(III) are adsorbed by potato starches. AA gives a total number of metal ions adsorbed in the structure, whereas EPR allows recognizing various types of Me-starch complexes and determines their electronic structure.

A relation between metal ion contents in the solution and concentration in the starches determined by AA is shown in Fig. 2. Up to 4 mg/g in solution a linear relation exists between concentration of paramagnetic salts in the solution and concentration of adsorbed metal ions in the starches both for Cu(II) and Fe(III) and sorption efficiency is almost 100% for the LUBOX but lower for the others. For higher concentrations a saturation effect appears which shows that a further increase in metal salt concentration in the solution results in constantly
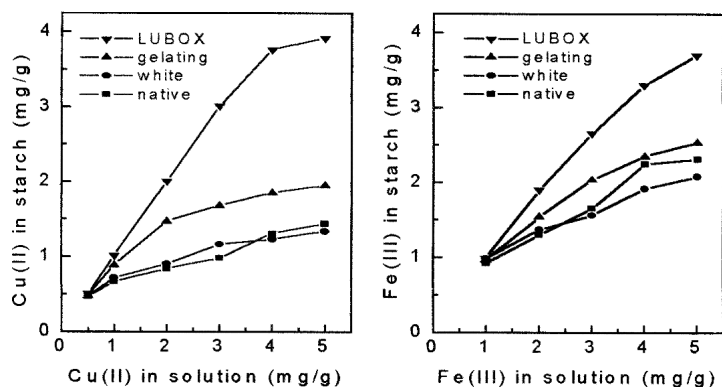

Fig. 2. Copper(II) (on the left) and iron(III) (on the right) adsorption in the studied starches. The ion contents were determined from atomic absorption studies.

diminishing sorption efficiency. The highest ion contents are clearly seen for Cu(II) and Fe(III) in LUBOX starch which is characterized by the highest degree of oxidation.

The similar relations we have found between concentration in solution and total intensity of EPR spectra both for Cu(II) and Fe(III). However, the spectra show that different forms of metal complexes exist in the starches. Representative EPR spectra of Cu(II) and Fe(III), recorded at room temperature, in white starch and LUBOX starch, respectively, are presented in Fig. 3.
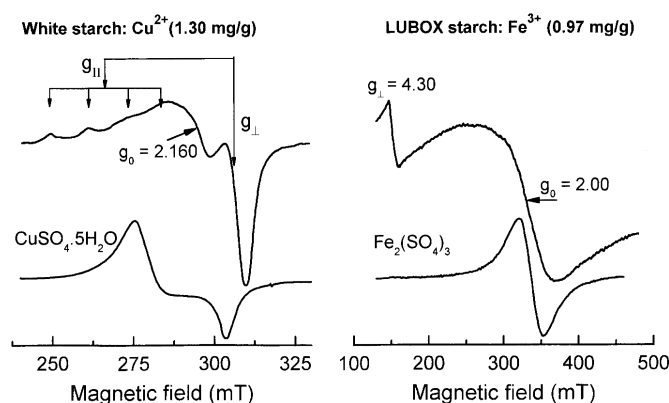

Fig. 3. Representative EPR spectra of Cu(II) and Fe(III) in potato starches compared with EPR spectra of paramagnetic salts which were used in mother solution as source of paramagnetic ions. The spectra were recorded at room temperature at the frequency of: 8.95 GHz for Cu(II) and 9.40 GHz for Fe(III).

The Cu(II) spectrum consists of two components. The first component is a typical EPR spectrum of individual Cu(II) complexes with resolved quartet of the hyperfine structure. Computer simulations show that the spectrum is described by spin Hamiltonian with axial symmetry of g and A tensors with parameters: $g_\parallel = 2.373(2)$, $g_\perp = 2.080(5)$, $A_\parallel = 12.1(1)$ mT, $A_\perp \leq 1.0(2)$ mT and Lorentzian line shape with linewidth of 6 mT at parallel complex orientation and 8 mT at perpendicular orientation [11]. The hyperfine quartet at parallel orientation and g-factor positions are marked by arrows in Fig. 3. The parameters indicate the $d_{x^2-y^2}$ ground state of Cu(II) typical of octahedral or square-pyramidal stereo-chemistry with coordination of copper ions by oxygen atoms similarly to Cu(II) in zeolites [12].

The second component of experimental spectrum is an isotropic spectrum without hyperfine structure located at $g_0 = 2.16$ with a peak-to-peak linewidth of 9 mT. This spectrum can be due to clusters (dimers, trimers ...) of copper(II) complexes coupled by exchange and magnetic dipolar interaction or can result from fast dynamics of individual Cu(II) complexes.

There is no trace of $CuSO_4 \cdot 5H_2O$ EPR line in the experimental spectrum (see the spectrum in Fig. 3, bottom). Thus, all copper ions introduced into the starch granules are coordinated to the starch molecules with no inclusions of the used copper salt. The results of paper [1] are not confirmed by our results because they used a much higher Cu(II) concentration and as the result the non-informative EPR spectra (with not resolved hyperfine structure) were recorded with effective g-factors: $g_\| = 2.441$, $g = 2.0023$. The spectra were due mainly to inclusions of a paramagnetic salt (like $CuSO_4 \cdot 5H_2O$).

The Fe(III) ions EPR spectrum in LUBOX starch consists of at least three components. The narrow line at low field ($g = 4.3$) is characteristic of individual Fe(III) complexes having effective rhombic local symmetry when zero field splitting D is much higher than the Zeeman splitting ($D \gg h\nu$). In such a case the effective g-factors are: $g_\| = 9.7$, $g_\perp = 30/7 = 4.3$. The nearly isotropic line at $g_\perp = 4.3$ origins from the transitions in the middle doublet of high-spin ($S = 5/2$) Fe(III) ions. The parallel orientation line at $g_\| = 9.7$ line is due to transitions in the lower and upper doublets of rhombic ($E > D$) high-spin Fe(III), where E is rhombic and D is the axial zero-field splitting parameters [13]. This line is usually broad, very week and located at around 60 mT, and hardly ever can be detected in a powder EPR spectrum. These components of the spectrum appear only for some starches with a high oxidation degree.

The second component of the Fe(III) spectrum is the broad asymmetrical line centred at around $g = 2.0$ with a linewidth about 30 mT. This line is characteristic of multinuclear clusters with distributed EPR parameters. The last component is the very broad (100–600 mT, depending on the starch) background line from centres with low symmetry and strongly distributed D and E parameters. The EPR line from the used iron sulphate $Fe_2(SO_4)_3$ is shown for comparison below the LUBOX : Fe(III) spectrum. This line of the 24 mT linewidth (characteristic of the exchange narrowing) is located at the same magnetic field as the cluster Fe line and it is hard to distinguish if the $Fe_2(SO_4)_3$ signal contributes to the cluster line or not.

In Fig. 4 a collection of selected EPR spectra (for more extended collection of the spectra see paper [11]) is presented for Fe(III) and Cu(II) in various potato starches with different paramagnetic ion contents.

In all starches with Fe(III) the line from clusters dominates and only in starches with high oxidation degree (CH_2O into COOH) the line from individual complexes is observed, i.e. for LUBOX (0.5% COOH), and for gelating starch ("Gel-forming food starch — 0.1% COOH) and high Fe concentration. In all samples the broad background line is visible. Thus, an adsorption of Fe(III) by potato starches prefers forming clusters of metals but not individual complexes except the case of high oxidation, i.e. high contents of weakly coordinating COOH groups.
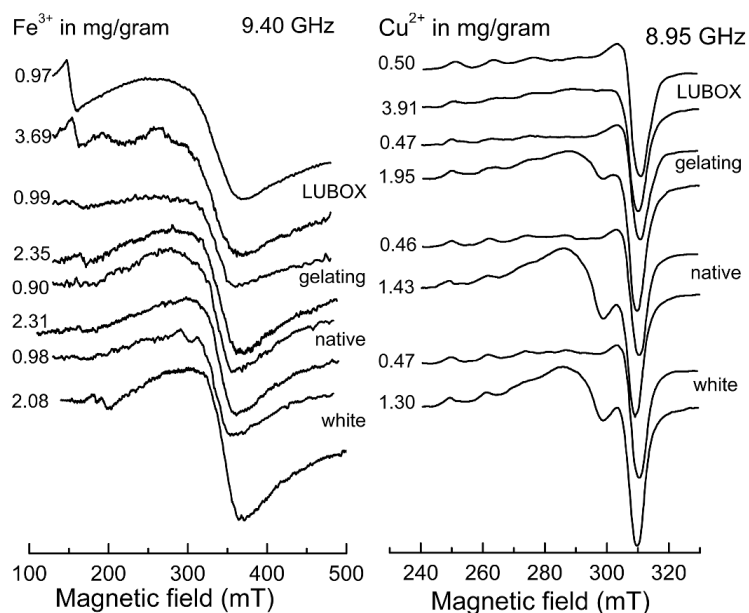
Fig. 4. Collection of selected room temperature EPR spectra for Fe(III) (left) and Cu(II) (right) in various starches with different ion contents.

A different behaviour is displayed by Cu(II). Individual complexes dominate for small concentrations and even for highest Cu(II) contents in LUBOX starch no clustering was observed. The line from Cu(II) clusters appears, however, for native and white starches ("Pudding powder") for Cu(II) concentration higher than about 0.9 mg/g, and for higher oxidized gelating starch for concentrations higher than about 2 mg/g. It means that in contrast to Fe(III) the Cu(II) ions are mainly selectively bonded in the starch structures.

We suggest that in native starch with low copper concentration (below 0.6 mg/g) the copper(II) ions are bonded by $-CH_2OH$ groups located outside the amylose helix. An increase in the copper contents leads to additional coordination by $-OH$ groups which are located inside the helix where clustering of the copper(II) complexes is preferred. Oxidation of starches resulting in an increase of easily coordinating bidentate $-COOH$ groups (replacing $-CH_2OH$ groups) leads to an increase in copper(II) ions in the outside helix positions where possible bridges between amylose chains can be formed.

General conclusion is that although both Cu(II) and Fe(III) can be introduced into potato starch structure, only Cu(II) is bonded selectively and does not display a tendency to clustering especially in oxidized samples. Moreover, we can convincingly claim that there does not exist inclusions of $CuSO_4 \cdot 5H_2O$ crystals whereas the existence of inclusions of $Fe_2(SO_4)_3$ cannot be ruled out on the basis of the observed EPR spectra.

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