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Effect of mineral elements on physicochemical properties of oxidised starches and generation of free radicals

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ABSTRACT

The objective of this study was to determine the effect of enrichment of oxidised starches with mineral compounds on their physicochemical properties and capability for free radical generation. Potato and spelt wheat starches were oxidised with sodium hypochlorite and, afterwards, modified with ions of potassium, magnesium and iron. The modified starches were analysed for: content of mineral elements, colour parameters ($L^*a^*b^*$), water binding capacity solubility in water at temperature of 50 and 80 °C, and susceptibility to enzymatic hydrolysis with α-amylase. In addition, thermodynamic characteristics of gelatinisation was determined by differential scanning calorimetry (DSC), and the number and character of thermally generated free radicals was assayed using electron paramagnetic resonance (EPR). Based on the results achieved, it was concluded that the quantity of incorporated minerals and changes in the assayed physicochemical parameters depended not only on the botanical type of starch but also on the type of the incorporated mineral element.

1. Introduction

Mineral elements constitute a group of chemical compounds indispensable for proper body functioning. In a human's body, they serve building functions, control metabolic processes, ionic balance and cell pressure, and sustain neuromuscular excitability. Owing to a lack of appropriate enzymatic systems, the minerals are not synthesised in a human body and have to be provided with food (Frossard, Bucher, Machler, Mozafar, & Hurrell, 2000; Soetan, Olaiya, & Oyewole, 2010). Nowadays, due to the contemporary lifestyle, demands for micro- and macroelements supplied with food products are observed to increase. Hence, producers are in the constant search for food components that would not only increase the sensory values of food, but would additionally be good carriers of deficit substances to the human body, including mineral elements (Śmigielska & Lewandowicz, 2007).

Starch is one of the natural and renewable raw materials abundant in nature. Owing to its specific structure, it easily interacts with different chemical substances, which results in the formation of modified starches with desirable physicochemical properties. For this reason, native and modified starches are one of the most multifunctional raw materials applied in the food industry (Tharanathan, 2005). One of the chemical methods of starch modification is its oxidation, which results in the formation of carbonyl and carboxyl groups. Their presence in oxidised starch enables better binding of metal ions (Kweon, Choi, Kim, & Lim, 2001; Śmigielska, Lewandowicz, Goslar, & Hoffman, 2005). Thereby, it is feasible to apply oxidised starch as a carrier of micro- and macroelements in a human diet, thus preventing metabolic diseases that result from the intake of excessively processed foods or from ill-balanced diets (Śmigielska & Le Thanh-Blicharz, 2010; Śmigielska, Lewandowicz, & Walkowski, 2005). This affords great opportunities for food producers and facilitates the production of foods enriched with mineral elements deficient to human. Owing to their physicochemical properties, the oxidised starches are integral constituents of creams, puddings, whipped cream, powder cake mixtures, sprinkles and coatings (Wurzburg, 1987).

Nevertheless, the presence of mineral elements incorporated to oxidised starches affects not only an increase in its nutritive value but also its physicochemical properties. Hence, the objective of this study was to determine the impact of mineral elements on physicochemical properties of oxidised starches and their capability for free radicals generation.

2. Materials and methods

2.1. Materials

The starch used for the study was potato starch produced by PPS Pepees S.A. (Łomża, Poland) and spelt starch isolated with the

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laboratory method from ECCO type 700 spelt wheat flour (Młyn Gospodarczy, Poland).

Oxidation was carried out following a procedure described by Forsell, Hamunen, Autio, Suorti, and Poutanen (1995). The modification was conducted in a 40% water suspension of starch, using NaClO (POCh, Poland) in amounts equivalent to 10 g Cl per kg of starch. The solution with pH = 10 was stirred for 50 min at temperature 20 ± 0.5 °C, neutralised to pH = 7, stirred and washed. Then, the starch samples were pulverised, dried and sieved.

The starches were modified by reactions with potassium, magnesium and iron ions. Potassium, magnesium and iron were incorporated into starch according to the following procedure. 100 g of starch were mixed with 250 cm^3 of water for 5 min and then the resulting dispersion was filtered through a filter funnel. The starch was mixed for 5 min with 200 cm^3 of the mixture of 1% (w/w) KCl and 0.05 M KOH in the volume ratio of 1:1 and of pH 12 or with 200 cm^3 of the mixture of 1% (w/w) $MgCl_2$ and saturated solution of $Mg(OH)_2$ in the volume ratio of 1:1 and of pH 8.5, or 1% (w/w) $FeSO_4$ respectively, for potassium, magnesium and iron. The resultant product was filtrated through a filter funnel and again 200 cm^3 of the mixture of potassium or magnesium salts and hydroxides or iron salt were added to the retentate, and then the sample was mixed for 5 min and filtrated. This action was repeated once again. The resultant starch preparation was rinsed with distilled water until chloride ions were absent, for modification with iron until sulphur ions were absent. Then, the starch samples were pulverised, dried and sieved.

2.2. Effectiveness of the oxidation process, potassium (K), magnesium (Mg) and iron (Fe) content

Oxidised starches were examined for: the carboxyl group content according to the Standard method ISO 11214 (1996), carbonyl group content according to Whistler, BeMiller, and Paschall (1967), and potassium (K), magnesium (Mg) and copper (Cu) contents by Atomic Absorption Spectroscopy using an Avanta Sigma spectrometer (GBC, Australia) with an air-acetylene burner, measured at λ = 324 nm. Prior to measurement, starch was mineralised in a mixture of nitric acid and sulphuric acid at a temperature of 250 °C, using a Wet Digester B-440 (Büchi, Switzerland).

2.3. Colour parameters

Colour parameters of the samples before and after heating (at temperature 150 °C 30 min, and 210 °C 30 min) were determined in the CIE $L^*a^*b^*$ system (measuring geometry d/8°, illuminant D65, observer 10°) using a Colour i5 spectrophotometer (X-Rite, USA). Total colour difference (ΔE) between oxidised and oxidised and modified with metals starches was calculated according to the equation:

$$\Delta E = \sqrt{(\Delta L^2) + (\Delta a^2) + (\Delta b^2)}$$

2.4. Water-binding capacity and solubility in water

Water-binding capacity and water solubility at temperatures of 50 and 80 °C were determined by the modified method of Leach (Richter, Augustat, & Schierbaum, 1968). A 1.25% (w/v) starch suspension was heated during 30 min at temperatures 50 and 80 °C and after this, the starch paste produced was recovered by mild centrifugation (2500 × g, ×15 min). Water binding capacity was calculated on dry mass of starch and solubility was based on soluble starch.

2.5. Susceptibility to enzymatic hydrolysis

The susceptibility to enzymatic hydrolysis was determined using α-amylase type VI-B, from porcine pancreas, activity 28 u/mg solid (Sigma–Aldrich, USA) following the method by Hung and Morita (2005) with own modification. To 40 mg of starch were added 38 cm^3 of water and 2 cm^3 solution (10 mg of α-amylase in 10 cm^3 0.05 M NaCl) of enzyme. The enzymatic hydrolysis of starch was maide at temperature 37 °C. Maltose content after hydrolysis was measured based on a colour reaction with 3,5-dinitrosalicylic acid after 20, 40, 60, 80, 100, and 120 min using a spectrophotometer. The extent of starch hydrolysis was presented as the percentage content of maltose in starch.

2.6. Thermodynamic characteristics of gelatinisation by DSC

The thermodynamic characteristics of gelatinisation were determined by using a differential scanning calorimeter DSC 204F1 (Phoenix Netsch, Germany). A starch–water (1:3) mixture was added to aluminium calorimetric cells, and left to stand for 24 h. Then, the samples were heated at temperatures in the range of 20–100 °C at a rate of 10 °C/min. An empty identical calorimetric cell was used as a reference. The temperatures at onset (T_o), peak (T_p), and end (T_e) of transition, and the enthalpy of transition related to the weight of starch (ΔH, J/g d.m.) were read from the thermograms.

2.7. EPR measurements

Samples of starch (about 20 mg) were placed in the quartz EPR tubes and heated in an oven upon air for 30 min at 423 K, followed by 30 min at 483 K. Before recording the EPR spectra, the tubes with the samples were cooled to a room temperature and closed with a paraffin membrane. Samples of starch enriched with iron ions were also studied without thermal treatment. EPR measurements were carried out on an X-band Bruker ELEXSYS 500 spectrometer (Karlsruhe, Germany) with 100 kHz field modulation. The spectra were recorded at 293 K with modulation amplitude of 0.5 mT and 0.1 mT before and after thermal treatment of the samples. They were registered at microwave powers: 10.0 and 3.0 mW. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was used as a g factor standard and as a reference sample (with the number of spin equal to 1.07×10^{15}/g) for the estimation of radicals number in the samples. The maximum error of the quantitative measurements was 0.05×10^{15} spin/g.

EPR parameters such as g-factor value, hyperfine splitting constant A and peak-to-peak line width ΔB_{pp} were found by a simulation procedure, using the program SIM 32 (Spałek, Pietrzyk, & Sojka, 2005). The accuracy of determination of EPR parameters was ±0.0005 for g values and ±0.1 mT for parameters A of radical signals, whereas g factor of iron species was estimated with the accuracy of 0.02.

2.8. Statistical analysis

The significance of differences between the values of the parameters was assessed by using the one-way analysis of variance and the Tukey test at a significance level $\alpha = 0.05$.

3. Results and discussion

3.1. Effectiveness of the oxidation process, potassium (K), magnesium (Mg) and iron (Fe) contents

After oxidation, starches contained respectively, 0.035 ± 0.002% (potato starch) and 0.012 ± 0.001% (spelt starch) of carboxyl groups

Table 1
Content of potasium, magnesium, iron and copper in oxidised starch before and after modification.

Starch	Potassium (mg/100 g d.m.)	Magnesium (mg/100 g d.m.)	Iron (mg/100 g d.m.)
Potato			
Oxidised	1.31 ± 0.01	2.64 ± 0.16	–
Oxidised and modified with K	183.09 ± 0.32	–	–
Oxidised and modified with Mg	–	27.21 ± 0.60	–
Oxidised and modified with Fe	–	–	99.87 ± 3.14
$LSD_{0.05}$	0.96	1.9	–
Spelt wheat			
Oxidised	0.13 ± 0.04	0.95 ± 0.07	–
Oxidised and modified with K	45.51 ± 1.99	–	–
Oxidised and modified with Mg	–	7.40 ± 0.21	–
Oxidised and modified with Fe	–	–	38.42 ± 2.15
$LSD_{0.05}$	4.96	0.77	–

–, below limit of detection. $LSD_{0.05}$, least significant difference at $\alpha = 0.05$.

as well as 0.020 ± 0.002% (potato starch) and 0.006 ± 0.001% (spelt starch) of carbonyl groups. Owing to the presence of carboxyl groups, the oxidised starches are capable of attaching metal ions to polysaccharide chains, thus forming a metal–starch complex (Łabanowska et al., 2008). For this reason, the oxidised starches containing more carboxyl groups display higher capability for binding metal ions. This is confirmed by results collated in Table 1 which shows that after modification, the oxidised potato starch contained more incorporated mineral elements than the spelt one. In addition, the presence of orthophosphoric(V) acid in potato starch affected more effective incorporation of mineral compounds. Oxidised potato starch contained 67 mg/100 g of phosphorus, that resulted in the presence of orthophosphoric(V) acid residues in this starch. Therefore more effective incorporation of mineral elements (potassium: over 4-fold, magnesium: over 3.5-fold, iron: over 2.5-fold) in oxidised potato starch than in oxidised spelt starch was also caused by the presence of orthophosphoric(V) acid residues which have high ability metal ions binding. The quantity of an incorporated mineral elements depended not only on the botanical origin of starch but also type of mineral element. Out of the metal ions used in the study, potassium was the one that was donated in the highest quantities in both potato and spelt starch (Table 1). Better reactivity of this element, compared to the other minerals examined (magnesium and iron), was likely to be due to its valence.

3.2. Colour parameters

Owing to the fact that the analysed starches were modified with metal ions that may affect the colour of resultant products, colour measurements of modified starch preparations were carried out and their results were presented in Table 2. All modifications with metals caused a decrease in lightness value (L^*) of the oxidised starches. The greatest decrease in the brightness of oxidised potato starch was caused by iron ions, whereas in the oxidised spelt starch – by magnesium ions. All analysed starches were characterised by negative values of a^* parameter. This indicates the contribution of the green component in the colour of all starches. In the case of this parameter, only modification with iron ions caused in both starches statistically significant changes compared to the oxidised starches. Positive values of b^* parameter point to the contribution of the yellow component in starch colour. After modification of both starches, ions of potassium and magnesium did not cause any significant changes in the value of this parameter. In turn, a tangible increase (over 5-fold) in the contribution of the yellow component of colour was noted for both starches enriched with iron ions. The yellow colour is typical of Fe(III) ion complexes, which may suggest the presence of trivalent iron in the modified starch. The value of ΔE^* parameter (total colour difference) calculated for potato starch enriched with iron and for spelt starch enriched with magnesium and iron was above 3.5, which pointed to significant colour differences. The other starches enriched with metal ions were characterised by the ΔE^* value below 2, which indicated a lack of differences in the colour of these starches compared to the oxidised starches (Francis & Clydesdale, 1975). The colour parameters determined for potato starch enriched with potassium and magnesium did not diverge from recommendations of the Polish Standard for potato starch (PN-A:74710, 1993), according to which the lightness parameter assayed in the $L^*a^*b^*$ system should account for not less than $L = 91$. In oxidised starches enriched with iron, Śmigielska, Lewandowicz, and Walkowski (2005) also noted a decrease in lightness parameter of modified starches that was found to depend on the quantity of incorporated metal. Results achieved in the present study confirm findings of those authors (Śmigielska, Lewandowicz, & Walkowski, 2005), that iron present in oxidised starch has a great

Table 2
Colour parameters of oxidised starch before and after modification.

Starch	Colour parameters			
	L^*	a^*	b^*	ΔE
Potato				
Oxidised	93.56 ± 0.03	−0.31 ± 0.02	2.14 ± 0.04	Reference
Oxidised and modified with K	91.87 ± 0.03	−0.32 ± 0.03	2.16 ± 0.03	1.69 ± 0.01
Oxidised and modified with Mg	92.02 ± 0.01	−0.31 ± 0.02	1.26 ± 0.04	1.76 ± 0.02
Oxidised and modified with Fe	89.09 ± 0.05	−1.01 ± 0.09	12.02 ± 0.02	10.87 ± 0.02
$LSD_{0.05}$	0.08	0.13	0.08	0.05
Spelt wheat				
Oxidised	94.21 ± 0.03	−0.38 ± 0.04	1.70 ± 0.03	Reference
Oxidised and modified with K	92.83 ± 0.04	−0.31 ± 0.04	2.28 ± 0.03	1.50 ± 0.04
Oxidised and modified with Mg	90.24 ± 0.01	−0.32 ± 0.01	1.06 ± 0.01	4.02 ± 0.02
Oxidised and modified with Fe	91.33 ± 0.05	−0.08 ± 0.01	8.79 ± 0.03	7.66 ± 0.02
$LSD_{0.05}$	0.06	0.08	0.07	0.09

$LSD_{0.05}$, least significant difference at $\alpha = 0.05$.

Table 3
Colour parameters of oxidised starch before and after modification after thermal treatment.

Starch	Colour parameters			
	L^*	a^*	b^*	ΔE
Potato				
Oxidised	90.52 ± 0.06	1.08 ± 0.04	11.52 ± 0.11	Reference
Oxidised and modified with K	79.66 ± 0.11	4.05 ± 0.06	1888 ± 0.32	181.03 ± 5.42
Oxidised and modified with Mg	93.99 ± 0.15	−0.99 ± 0.03	5.66 ± 0.11	50.22 ± 0.66
Oxidised and modified with Fe	78.81 ± 0.06	1.44 ± 0.10	15.58 ± 0.03	153.78 ± 2.23
$LSD_{0.05}$	0.26	0.16	0.47	8.91
Spelt wheat				
Oxidised	95.91 ± 0.13	−0.61 ± 0.04	4.45 ± 0.13	Reference
Oxidised and modified with K	91.23 ± 0.08	1.18 ± 0.04	9.10 ± 0.17	46.78 ± 1.82
Oxidised and modified with Mg	92.59 ± 0.14	−0.96 ± 0.07	4.55 ± 0.11	11.22 ± 1.78
Oxidised and modified with Fe	88.96 ± 0.22	0.32 ± 0.06	11.99 ± 0.13	105.90 ± 2.25
$LSD_{0.05}$	0.39	0.14	0.36	5.12

$LSD_{0.05}$, least significant difference at $\alpha = 0.05$.

impact on a change in its colour. The thermally treated starch preparations showed decreased values of L^* parameter, with the decrease being the greatest in the starches donated with potassium and iron (Table 3). Starch heating resulted also in changes of the a^* value from negative in the oxidised starches to positive in thermally activated starches, except for starches donated with magnesium and the non-donated oxidised spelt starch. This points to an increase in the red component. All starches subjected to the thermal treatment showed also an increase in b^* parameter that was especially tangible in starches donated with iron and potassium, which indicates a significant increase in the yellow component. Worthy of notice is the fact that the changes in the b^* parameter are more intensive in potato starch, which confirms better binding of metal ions with starch matrix. In addition, it seems significant that the increase in yellow component was the greatest in starches donated with potassium and not – as expected – in these donated with iron which under conditions of higher temperature should be subject to relatively intensive oxidation to Fe(III) ions. As shown by EPR analyses (discussion below), during thermal treatment of starch, carbohydrate radicals are being generated and in the samples enriched with iron ions the Fe(III) ions are concomitantly reduced to Fe(II) ions.

3.3. Water-binding capacity and solubility in water

The water-binding capacity and solubility in water of starches at temperatures of 50 and 80 °C are presented in Table 4. These temperatures were selected based on onset (T_o) and end (T_e) gelatinisation temperatures determined with the DSC (Table 4). In the oxidised potato starch, the water-binding capacity at a temperature of 50 °C was changing only after modification with potassium ions (over 3-fold increase), whilst the other modifications did not elicit significant changes in the value of this parameter. At a temperature of 80 °C, the oxidised potato starch was completely soluble in water, thereby there was no possibility of determining its water-binding capacity. That property of this starch remained unchanged after the applied modifications with metal ions. In turn, potato starch enrichment with potassium and iron had no effect on its solubility in water at a temperature of 50 °C. The modification with these elements caused a significant increase in its solubility, compared to the non-enriched starch and that modified with magnesium. The enrichment of oxidised spelt starches with particular mineral elements caused an increase in their water-binding capacity at 50 °C. An opposite effect was observed at a temperature of 80 °C, for modification of this starch with the applied minerals did not cause statistically significant changes in the value of this parameter. The solubility of spelt starch in water at temperatures of 50 and 80 °C after its enrichment with metals increased and was the highest after modification with magnesium ions at both temperatures of assay. The modification with both potassium and iron caused an increase in starch solubility to a similar level at respective temperatures. The water-binding capacity of starch is determined, most of all, by amylopectin, whereas its solubility – by amylose (Tester & Morisson, 1990). In turn Kuakpetoon and Wang (2008) demonstrated that in starch not only amylose chains but also the amylopectin ones are subject to oxidation. Hence, the presence of carboxyl group in amylopectin as well as that of new substituents in the form of metal ions weaken of intramolecular bonds and easier penetration of water to starch granule interior. The increased solubility of potato and spelt starches in water was, in turn, caused by the process of amylose depolymerisation that might proceed during starch modification with metals.

Table 4
Water binding capacity of oxidised starch before and after modification.

Starch	Water binding capacity (g/g d.m.)		Solubility in water (%)	
	50 (°C)	80 (°C)	50 (°C)	80 (°C)
Potato				
Oxidised	2.87 ± 0.04	X	2.97 ± 0.18	100
Oxidised and modified with K	9.47 ± 0.67	X	9.53 ± 0.50	100
Oxidised and modified with Mg	2.97 ± 0.26	X	2.58 ± 0.26	100
Oxidised and modified with Fe	2.68 ± 0.01	X	4.01 ± 0.03	100
$LSD_{0.05}$	1.21	–	1.03	–
Spelt wheat				
Oxidised	3.90 ± 0.06	8.15 ± 0.06	1.95 ± 0.27	6.22 ± 0.16
Oxidised and modified with K	4.55 ± 0.35	7.56 ± 0.05	7.66 ± 0.35	14.38 ± 0.11
Oxidised and modified with Mg	6.13 ± 0.21	8.93 ± 0.16	12.49 ± 0.58	21.01 ± 0.51
Oxidised and modified with Fe	5.25 ± 0.28	8.23 ± 0.01	6.14 ± 0.17	14.45 ± 0.60
$LSD_{0.05}$	0.61	n.s.	1.36	1.01

X, samples was completely dissolved; n.s., not significant; $LSD_{0.05}$, least significant difference at $\alpha = 0.05$.

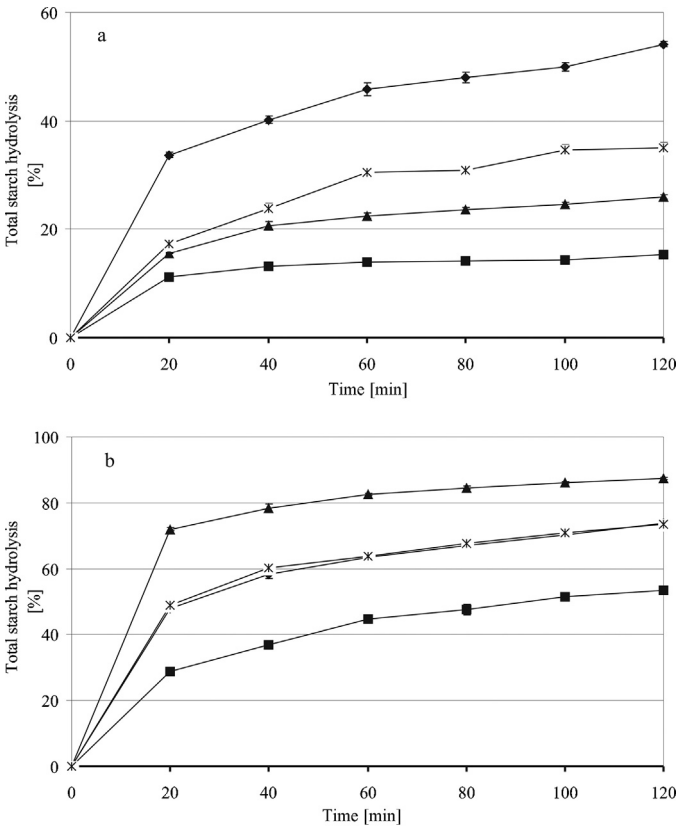

Fig. 1. Susceptibility to α amylase oxidised starches before and after modification. (a) Potato, (b) spelt wheat, (■) oxidised; (♦) oxidised and modified with K; (▲) oxidised and modified with Mg; (Ж) oxidised and modified with Fe.

3.4. Susceptibility to enzymatic hydrolysis

Fig. 1a and b depict the susceptibility of the modified starches to enzymatic hydrolysis with α-amylase. The oxidised potato starch was significantly less susceptible to enzymatic hydrolysis within 120 min than the oxidised spelt starch. Grains of potato starch are characterised by type B crystallinity which causes greater resistance to the action of enzymes, compared to cereal starches that are characterised by type A crystallinity (Jane, Wong, & McPerhson, 1997). The applied modifications with metals increased the susceptibility of the enriched starches to α-amylase, yet it depended not only on the botanical origin of starch but also on the type of metal used for modification. Out of the potato starches examined, the greatest susceptibility to enzymatic hydrolysis was demonstrated by the starch enriched with potassium, whereas out of the spelt starches – by that enriched with magnesium. The oxidised spelt starch enriched with potassium or iron was equally susceptible to hydrolysis with α-amaylse in the entire measurement range. The enhanced susceptibility of the enriched starches to enzymatic hydrolysis was due to the loosening of intramolecular bonds that resulted from the presence of metal ions in starch structure. Owing to this, amylase had a better access to α-1,4-glycosidic bonds contained in amylose and amylopectin. The greater susceptibility of potato starch enriched with potassium and iron and of spelt starch enriched with magnesium to enzymatic hydrolysis was also due to their higher water-binding capacity and better solubility in water (priori to gelatinisation), compared to the other starches (Table 3). In addition, during modification, disintegration and drying of starch, small cracks and enlargement of pores occurring in the external structure of starch grain could occur, thus increasing the contact surface with the enzyme (Yan & Zhengbiao, 2010). Östergard, Bjorck, and Narsson (1998) and Chung, Shin, and Lim (2008) demonstrated that oxidised and hydroxypropylated starches were more susceptible to the action of α-amylase at the initial stage of hydrolysis (up to ca. 60 min) than the native starches. According to those authors, this was due to degradation of starch granules upon the modification process.

3.5. Thermodynamic characteristic of gelatinisation by DSC

Results of thermodynamic characteristics of gelatinisation determined with the DSC method were shown in Table 5. The applied processes of potato starch enrichment affected a decrease in T_o gelatinisation temperature of the resultant starches. The decrease of gelatinisation temperature was the greatest in the case of potassium–enriched starch. The other mineral elements decreased the value of this parameter to a similar level. In turn, in the case of spelt starch, a decreased gelatinisation temperature was noted upon starch enrichment with magnesium ions. After modifications with the other minerals, the decrease in the value of this parameter was statistically insignificant. The decrease of gelatinisation temperature observed for the enriched oxidised starches points to a lesser thermal stability of starch granules and better penetration of water to their interiors. It results from the deterioration of the internal structure with new substituents in the form of mineral compounds. Our previous study (Łabanowska et al., 2011) demonstrated that the presence of Cu(II) ions in oxidised starch affected the destabilisation of starch granule structure and generation of free radicals during starch heating. In the case of oxidised potato starch enriched with minerals, a high negative coefficient of simple correlation ($R = -0.89$) was determined between water-binding capacity at a temperature of 50 °C (Table 3) and temperature of the onset peak of gelatinisation (T_o). The applied modifications with metals had various effects on the other temperature parameters of the thermodynamic characteristics of gelatinisation and these effects were mainly affected by the botanical origin of starch. In the case of potato starch, only enrichment with potassium ions caused statistically significant changes in T_p and T_e values by decreasing them. In turn, in the case of spelt starch all mineral elements caused a significant decrease of these parameters (T_p and T_e), with the decrease being the greatest upon modification with magnesium. In potato starch, only modification with magnesium caused statistically significant changes in gelatinisation enthalpy (ΔH) by decreasing it. Different effects were observed in the case of spelt starch, for the value of gelatinisation enthalpy (ΔH) was reduced by all metals, with the greatest effect triggered by magnesium (over 60%). The decrease of starch gelatinisation enthalpy is due to destruction of double helices present inside amylopectin and constituting the crystalline forms of starch (Sandhu, Kaur, Singh, & Lim, 2008), which occurred as a result of carboxyl groups substitution with minerals in amylopectin. This theory is corroborated by findings of Śmigielska and Lewandowicz (2007) who demonstrated that the enrichment of oxidised potato starch with copper ions was reducing its crystallinity. Also Wootton and Bamunuarachchi (1979) claimed that each new substituent introduced to starch was reducing gelatinisation enthalpy by deteriorating the internal structure of starch.

3.6. EPR measurements

The oxidised potato and spelt starches before thermal treatment do not display paramagnetic centres. The centres appear already after incorporation of iron ions to starch. In an EPR spectrum registered at a room temperature, two signals appear: an anisotropic signal with a typical g factor of $g = 4.24$ and a wide, intensive signal with $g = 2.00$. They indicate explicitly the presence of Fe(III) ions in the oxide environment (Goodman, McPhail, & Linehan, 1986; Miller & Brudvig, 1999; Polovka, Brezová, & Staško, 2003; Sutter,

Table 5
Thermodynamic characteristics of gelatinisation of oxidised starch before and after modification.

Starch	T_o (°C)	T_p (°C)	T_e (°C)	ΔH (J/g d.m.)
Potato				
Oxidised	60.1 ± 0.2	66.8 ± 0.1	74.5 ± 0.3	11.52 ± 0.19
Oxidised and modified with K	56.2 ± 0.1	65.6 ± 0.1	73.3 ± 0.2	12.16 ± 0.24
Oxidised and modified with Mg	58.8 ± 0.3	66.4 ± 0.1	74.0 ± 0.2	10.42 ± 0.36
Oxidised and modified with Fe	58.4 ± 0.2	66.5 ± 0.4	74.2 ± 0.4	11.64 ± 0.31
$LSD_{0.05}$	0.5	0.5	0.7	0.74
Spelt wheat				
Oxidised	53.1 ± 0.3	61.9 ± 0.2	69.3 ± 0.2	6.32 ± 0.13
Oxidised and modified with K	53.1 ± 0.6	60.0 ± 0.2	67.4 ± 0.3	3.65 ± 0.37
Oxidised and modified with Mg	50.7 ± 0.3	57.9 ± 0.3	66.0 ± 0.3	2.26 ± 0.05
Oxidised and modified with Fe	53.7 ± 0.2	60.2 ± 0.1	67.7 ± 0.2	4.86 ± 0.12
$LSD_{0.05}$	0.9	0.5	0.6	0.53

$LSD_{0.05}$, least significant difference at $\alpha = 0.05$.

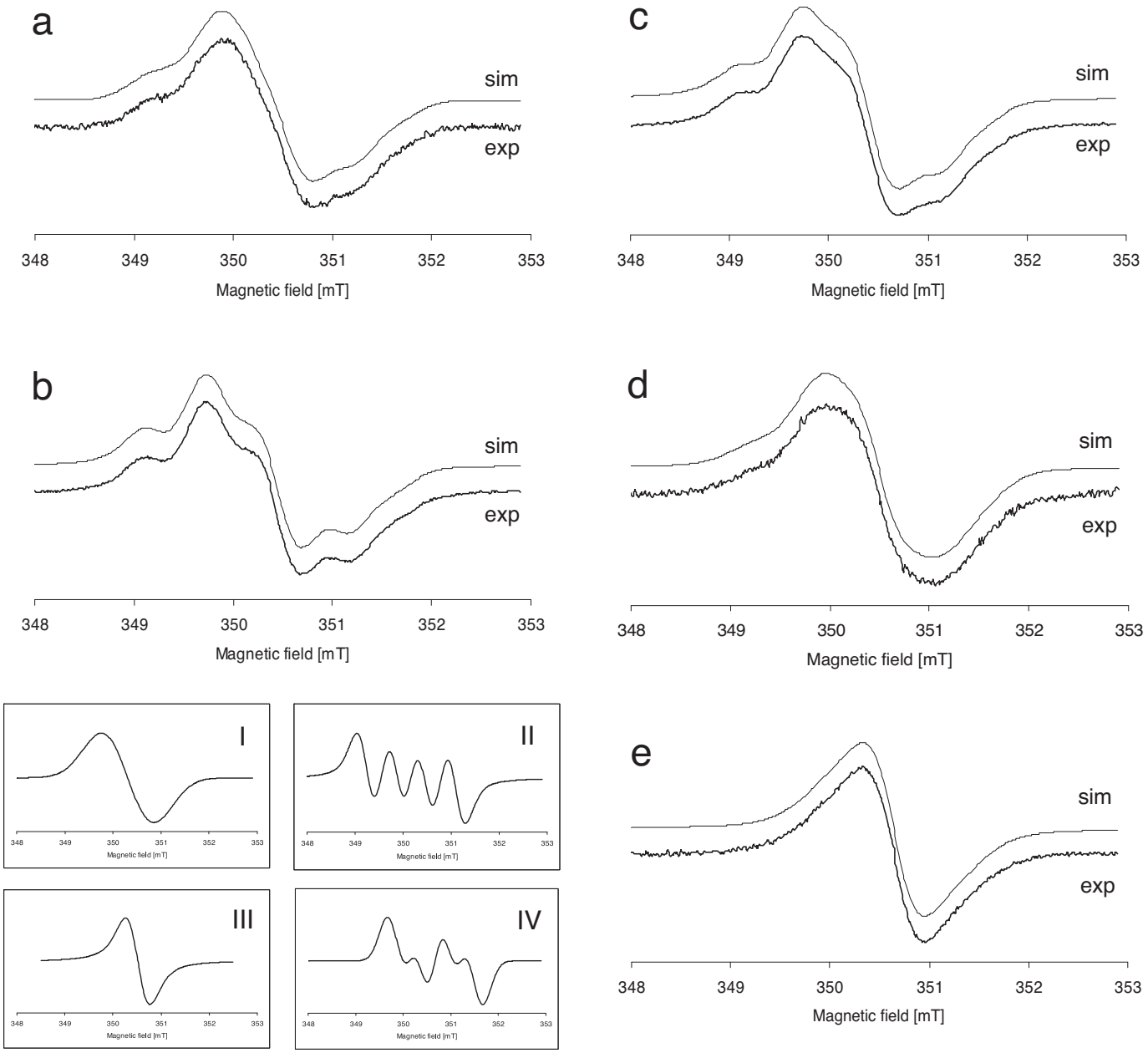

Fig. 2. The experimental (exp) and simulated (sim) spectra of oxidised starches registered at 293 K, at 3 mW, in the range 5 mT. (a) Oxidised spelt starch, (b) oxidised potato starch, (c) oxidised potato starch modified by K, (d) oxidised potato starch modified by Mg, (e) oxidised potato starch modified by Fe, I, II, III, IV particular signals used to the simulation of the spectra.

Table 6
The amount of radicals in thermally treated native and oxidised starch before and after modification.

Starch	Number of radicals (spins/g sample)
Potato	
Native	1.7×10^{15}
Oxidised	10.7×10^{15}
Oxidised and modified with K	16.8×10^{15}
Oxidised and modified with Mg	3.0×10^{15}
Oxidised and modified with Fe	13.1×10^{15}
Spelt wheat	
Native	3.2×10^{15}
Oxidised	2.1×10^{15}
Oxidised and modified with K	7.2×10^{15}
Oxidised and modified with Mg	1.6×10^{15}
Oxidised and modified with Fe	2.4×10^{15}

Wasowicz, Howard, Hossner, & Ming, 2002). The intensity of these signals depends on the type of starch and is significantly higher in the case of potato starch, thereby confirming more effective binding of iron ions with the matrix of this starch. The presence of signals of Fe(III) ions is a sound proof that Fe(II) are partly oxidised in the process of their incorporation to starch. The thermal activation of starch results in the appearance of signals with *g* factors in the range of 2.0030–2.0067 in the EPR spectra, i.e. in the range typical of radical centres (Fig. 2). In the case of starches donated with iron ions, the appearance of radical signals is accompanied by a decrease in the intensity of signal of iron ions, being more visible in potato starch. The decrease of signal intensity of Fe(III) ions indicates its reduction to Fe(II), which does not exhibit a signal in the conditions of EPR signal measurement. Such a dependency was also observed in the case of native starches: potato and maize ones, donated with iron ions (Łabanowska, Kurdziel, Bidzińska, Fortuna, et al., 2013). Analogous dependency was noted between the intensity of radical signal, generated as a result of heating potato starch donated with copper ions, and Cu^{2+} signal intensity, strongly decreasing as a result of reduction to diamagnetic Cu^{+} ions (Łabanowska et al., 2011).

The comparison of the intensities of radical signals demonstrates that starch oxidation enhances the process of thermal generation of radicals in potato starch, whereas inhibits slightly their generation in spelt starch. This difference can result from the contents of COOH and CHO groups created during oxidation with greater yield in potato starch. As it was shown in our previous studies (Łabanowska et al., 2008, 2011; Łabanowska, Kurdziel, Bidzińska, Wesełucha-Birczyńska, et al., 2013) the presence of COOH groups facilitates the radical formation, what clearly took place in potato starch. In the case of spelt starch, during oxidation three times smaller amounts of carbonyl and carboxyl groups were created. Both factors, oxidation as well as subsequent thermal treatment used to radical generation cause depolimerization of starch. Small contents of CHO and COOH groups in spelt starch indicate its resistance towards oxidation connected with more compact structures of cereal starches. Therefore, the process of depolimerization occurs less effectively leading to decrease of susceptibility towards thermal radical formation (Łabanowska, Kurdziel, Bidzińska, Wesełucha-Birczyńska, et al., 2013)

In the case of potato starch, in which oxidation causes ca. six-fold increase in the number of generated radicals (Table 6), four signals may be distinguished in the EPR spectrum. Their parameters, gathered in Table 7, have been discussed in details in our previous paper (Łabanowska, Kurdziel, Bidzińska, Fortuna, et al., 2013). In turn, in the spelt starch, in which oxidation results in a decreased number of generated radicals by ca. 60% compared to the oxidised starch, the simulation reveals the presence of three signals (Table 7). Signal I is ascribed to the radical that is generated by detachment of a hydrogen atom from a carbon atom C(1) of a glucose unit (Kuzuya, Yamauchi, & Kondo, 1999; Łabanowska et al., 2008, 2011). The magnetic moment of the unpaired electron localised on C(1) atom interacts with the magnetic moment of a hydrogen atom nucleus H_β linked with C(2) atom, thus leading to the formation of two lines of the hyperfine structure. The generation of signal II, exhibiting four-line hyperfine structure, is ascribed to the radical formed probably by the detachment of a hydrogen atom from C(6) (Łabanowska et al., 2011). The interaction of the magnetic moment of the unpaired electron localised on C(6) with magnetic moments of two non-equivalent hydrogen atoms H_α and H_β, is the reason of the appearance of four lines of the hyperfine structure. The III signal, that does not exhibit the hyperfine structure, is characterised by a significantly lower value of the *g* factor than signal I and II, which suggests that the electron is delocalised in the system of conjugated bonds carbon–carbon (Łabanowska, Wesełucha-Birczyńska, et al., 2013). A lack of signal III in the spectrum of the spelt starch proves that the process of radicals generation in this starch proceeds less effectively and its structure is more stable. Good fitting of the theoretical spectra to the experimental ones for both types of starches was achieved assuming the presence of an additional signal IV with a small contribution. This signal is characterised by the *g* factor in the range of 2.0030–2.0031 and six lines of the hyperfine structure and was initially ascribed to the radical generated by the detachment of the OH group from C(6) atom. In such a system, interactions may occur between magnetic moments of the unpaired electron with magnetic moments of two H_α atoms and one H_β atom.

The introduction of K, Mg and Fe ions to starch structure is observed to change both the number of generated radicals and the character of signals. These modifications are also dependent on the type of starch. The introduction of K ions does not affect the character of the observed signals in both types of starches, whereas it significantly increases the number of thermally generated radicals (Table 6). The opposite effect on the number of generated radicals is observed in the case of starch donated with magnesium ions (Table 6), that leads to a reduced number of radicals. The presence of Fe(III) ions is also observed to significantly modify parameters and character of the generated signals (Fig. 2). In the EPR spectrum, A and B signals appear with parameters other than those found for the radicals in the oxidised native starches, thus showing other localisation of the unpaired electron. Signal A, with splitting similar to that of signal I, displays a decrease of the *g* factor compared to the signals in the non-donated oxidised starches. In turn, signal B is in its characteristics similar to signal III. The mechanism of radicals generation in starches donated with iron is, probably, analogous to that described for starch donated with Cu ions (native and oxidised one) and presented in a work by Łabanowska et al. (2011). The donation with iron ions affects also the number of generated radicals. In both starches, the number of radicals is increasing. As mentioned above, this increase is due to a decrease in the intensity of Fe(III) signal and, as demonstrated in the study by Łabanowska et al. (2011), results from other mechanism of carbohydrate radicals generation than in the starches not donated with metal ions.

When analysing the effect of ions on the process of radicals generation on potato and spelt starches, it seems that the course of this process is determined by the oxidation state of ions. In the case when it might be subject to changes, the mechanism of the process is different than in the case of ions with constant valence. Of great significance seems also the size of an radius of particular metal ions introduced to starch, and its charge. The mono-positively charged potassium ion with an ionic radius almost two-fold greater than that of the other ions (Mg, Fe) is more easily bound by —COOH groups, and its incorporation presumably damages starch structure to a significantly greater extent, thus facilitating its degradation

Table 7
EPR parameters of spectra of oxidised starch before and after modification with Fe(II) salt.

Starch	Signal	g	A (mT)	Contribution (%)	Radical attribution
Oxidised potato starch	I	2.0054	0.5	55	C(1)
	II	2.0060	$A_1 = 0.6$, $A_2 = 1.2$	20	C(6)
	III	2.0041	–	16	Delocalized[a]
	IV	2.0031	$A_1 = 0.5$, $A_2 = 1.1$, $A_3 = 0.2$	9	C(6)
Oxidised potato starch + Fe	A	2.0042	0.4	59	C(1)
	B	2.0041	–	41	
Oxidised spelt starch	I	2.0057	0.5	76	C(1)
	II	2.0066	$A_1 = 0.6$, $A_2 = 1.2$	15	C(6)
	III	–	–		
	IV	2.0030	$A_1 = 0.4$, $A_2 = 1.0$, $A_3 = 0.2$	9	C(6)
Oxidised spelt starch + Fe	A	2.0046	0.4	61	C(1)
	B	2.0042	–	39	

[a] Delocalised on conjugated double bonds.

at higher temperatures. In addition, the interactions of metal ions with starch matrix depend to a significant extent on the type of starch. Potato starch with a higher amylose content, and thus with a less compact structure, is more easily oxidised, and additionally by possessing phosphate groups more effectively interacts with metal ions. The insignificant effect of Mg ions on the thermal generation of radicals may be caused by its lower content compared to the other ions, which results from the fact that two functional groups are necessary for its binding with a starch matrix.

4. Conclusions

Based on the results achieved, it was concluded that the number of incorporated mineral compounds as well as changes in the determined physicochemical parameters depended on the botanical origin of starch and the type of donated mineral element. The oxidised potato starch turned out to be more susceptible to enrichment with mineral elements than the oxidised spelt starch, whereas out of the minerals examined–potassium was the one that attached in the highest quantity to starch. The modifications of both starches with iron exerted the greatest effects on changes in the determined colour parameters. In the oxidised spelt starch, the enrichment with the applied minerals caused an increase in its water-binding capacity at a temperature of 50 °C and its solubility in water at temperature of 50 and 80 °C. The applied modifications with mineral elements affected also increased susceptibility to the action of α-amylase and changes in parameters of thermodynamic characteristics of gelatinisation.

The EPR method turned out especially useful for analyses of processes of thermal generation of carbohydrate radicals in starches of various botanical origin, additionally providing information on the structure of starch. It was demonstrated that the mechanism of radical generation and their number depended on the type of metal ion introduced to starch as well as on starch structure. The spelt starch was less susceptible to oxidation processes, which resulted in a lower number of radicals generated during its thermal treatment. The metals ions introduced to starch structure modified the course of thermal generation of radicals to a different extent depending on their charge, size of the ionic radius and possibility of changing the oxidation state. Iron ions, capable of changing the oxidation state, were causing a change in the mechanism of radicals generation, whilst di-positive magnesium ions – less easily interacting with functional groups of starch, were inhibiting the process of radicals generation, whereas the mono-positive potassium ions with a large ionic radius, by strongly interacting with starch matrix were facilitating its degradation at higher temperatures, thus leading to an increase in the number of thermally generated carbohydrate radicals.

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References

Chung, H. J., Shin, D. H., & Lim, S. T. (2008). In vitro starch digestibility and estimated glycemix index of chemically modified corn starches. *Food Research International*, *41*, 579–585.
Forsell, P., Hamunen, A., Autio, K., Suorti, T., & Poutanen, K. (1995). Hypochlorite oxidation of barley and potato starch. *Starch/Stärke*, *47*, 371–377.
Francis, F. J., & Clydesdale, F. M. (1975). *Food colorimetry: Theory and applications*. Westport: The AVI Publishing Company Inc.
Frossard, E., Bucher, M., Machler, F., Mozafar, A., & Hurrell, R. (2000). Potential for increasing the content and bioavailability of Fe, Zn and Ca in plants for human nutrition. *Journal of the Science of Food and Agriculture*, *80*(7), 861–879.
Goodman, B. A., McPhail, D. B., & Linehan, D. J. (1986). Oxygen-induced free radical in wheat roots. *Free Radical Research Communications*, *2*, 2173–2178.
Hung, P., & Morita, N. (2005). Physicochemical properties and enzymatic digestibility of starch from edible canna (*Canna edulis*) grown in Vietnam. *Carbohydrate Polymers*, *61*, 314–321.
Jane, J., Wong, K., & McPerhson, A. (1997). Branch structure differences in starches of A- and B- X-ray patterns revealed by their *Naegeli dextrins*. *Carbohydrate Research*, *300*, 219–227.
Kuakpetoon, D., & Wang, Y. J. (2008). Locations of hypochlorite oxidation in corn starches varying in amylose content. *Carbohydrate Research*, *343*, 90–100.
Kuzuya, M., Yamauchi, Y., & Kondo, S. (1999). Mechanolysis of glucose-based polysaccharides as studied by electron spin resonance. *Journal of Physical Chemistry B*, *103*, 8051–8059.
Kweon, D.-K., Choi, J.-K., Kim, E.-K., & Lim, S.-T. (2001). Adsorption of divalent metal ions by succinylated and oxidised corn starches. *Carbohydrate Polymers*, *46*, 171–177.
Łabanowska, M., Bidzińska, E., Dyrek, K., Fortuna, T., Pietrzyk, S., & Rożnowski, J. (2008). Cu^{2+} ions as a paramagnetic probe in EPR studies of radicals generated thermally in starch. *Starch/Stärke*, *60*(3–4), 134–145.
Łabanowska, M., Bidzińska, E., Pietrzyk, S., Juszczak, L., Fortuna, T., & Błoniarczyk, K. (2011). Influence of copper catalyst on the mechanism of carbohydrate radicals generation in oxidized potato starch. *Carbohydrate Polymers*, *85*, 775–785.
Łabanowska, M., Kurdziel, M., Bidzińska, E., Fortuna, T., Pietrzyk, S., Przetaczek-Rożnowska, I., et al. (2013). Influence of metal ions on thermal generation of carbohydrate radicals in native and modified starch studied by EPR. *Starch/Starke*, http://dx.doi.org/10.1002/star.201200165
Łabanowska, M., Kurdziel, M., Bidzińska, E., Wesełucha-Birczyńska, A., Pawcenis, D., Łojewski, T., et al. (2013). Influence of starch oxidation and phosphorylation on thermal generation of carbohydrate radicals studied by electron paramagnetic resonance. *Starch/Starke*, http://dx.doi.org/10.1002/star.201200164
Łabanowska, M., Wesełucha-Birczyńska, A., Kurdziel, M., & Sepioło, K. (2013). The mechanism of thermal activated radical formation in potato starch studied by EPR and Raman spectroscopies. *Carbohydrate Polymers*, *91*, 339–347.
Miller, A. F., & Brudvig, G. W. (1999). A guide to electron paramagnetic resonance spectroscopy of Photosystem II membranes. *Biochimica and Biophysica Acta*, *1056*, 1–18.
Östergard, K., Bjorck, I., & Narsson, A. (1988). A study of native and chemically modified potato starch Part. 1. Analysis and enzymatic availability *in vitro*. *Starch/Starke*, *40*, 58–66.
PN-A:74710. (1993). *Przetwory ziemniaczane. Skrobia ziemniaczana*. (in Polish).
Polovka, M., Brezová, V., & Staško, A. (2003). Antioxidant properties of tea investigated by EPR spectroscopy. *Biophysica Chemistry*, *106*, 39–56.
Richter, M., Augustat, S., & Schierbaum, F. (1968). . *Ausgewählte Methoden der Stärkechemie* (vol. 40) Leipzig: VEB Fachbuchverleg (in Germany).

Sandhu, K. S., Kaur, M., Singh, N., & Lim, S. T. (2008). A comparision of native and oxidized normal and waxy corn starches: Physicochemical, thermal, morphological and pasting properties. *LWT Food Science and Technology*, *41*, 1000–1010.

Śmigielska, H., & Le Thanh-Blicharz, J. (2010). Research on zinc fortified potato starch and on its use in dessert production. *ACTA Scientiarum Polonorum Technologia Alimentaria*, *9*(2), 217–226.

Śmigielska, H., & Lewandowicz, G. (2007). Właściwości funkcjonalne skrobi modyfikowanych wzbogacanych jonami miedzi. *Żywność, Nauka, Technologia Jakość*, *6*(55), 198–209 (in Polish).

Śmigielska, H., Lewandowicz, G., Goslar, J., & Hoffman, S. K. (2005). Binding of the trace elements: Cu(II) and Fe(III) to the native and modified nutritive potato starches studied by EPR. *Acta Physica Polonica A*, *108*, 303–310.

Śmigielska, H., Lewandowicz, G., & Walkowski, A. (2005). Wpływ dodatku mikroelementów na właściwości użytkowe skrobi utlenionych. Folia Universitatis. *Agriculturae Stetinensis Scientia Alimentaria*, *246*(4), 289–302.

Soetan, K. O., Olaiya, C. O., & Oyewole, O. E. (2010). The importance of mineral elements for humans, domestic animals and plants: A review. *African Journal of Food Sciences*, *4*(5), 200–222.

Spałek, T., Pietrzyk, P., & Sojka, Z. (2005). Application of genetic algorithm joint with Powell method to non linear least squares fitting of powder EPR spectra. *Journal of Chemical Information Modeling*, *45*, 18–29.

Standard ISO 11214. (1996). *Modified starch – determination of carboxyl group content of oxidized starch*.

Sutter, B., Wasowicz, T., Howard, T., Hossner, L. R., & Ming, D. W. (2002). Characterization of iron manganese and copper synthetic hydroxyapatites by electron paramagnetic resonance spectroscopy. *Soil Science Society of America Journal*, *66*, 1359–1366.

Tester, R. F., & Morisson, W. R. (1990). Sweeling and gelatinization of cereal starches I. Effect of amylopectin amylose and lipids. *Cereal Chemistry*, *67*, 551–557.

Tharanathan, R. N. (2005). Starch-value addition by modification. *Critical Reviews in Food Science and Nutrition*, *45*, 371–384.

Whistler, R. L., BeMiller, E., & Paschall, E. F. (1967). . *Starch: Chemistry and technology* (vol. 2) New York: Academic Press.

Wootton, M., & Bamunuarachchi, A. (1979). Application of differential scanning calorimetry to starch gelatinization. *Starch/Stärke*, *31*(6), 201–204.

Wurzburg, O. B. (1987). *Modified starches properties and uses*. Boca Raton Florida: CRS Press Inc.

Yan, H., & Zhengbiao, G. U. (2010). Morphology of modified starches prepared by different methods. *Food Research International*, *43*, 767–772.