

## EFFECT OF IRON IONS ON SELECTED PHYSICOCHEMICAL PROPERTIES OF OCTENYLSUCCINATE STARCH

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**Abstract.** Native maize starch was esterified using octenyl succinic anhydride (OSA) at four levels: 0%, 3%, 6%, 9% and, afterwards, enriched with iron ions. The aim of this work was to evaluate the effect of iron ion son the physicochemical properties of OSA starch. The modified starches were subjected to the following analysis: content of iron, water-binding capacity (WBC) and solubility in water (SW) at 50 and 60°C, colour parameters in the CIEL\*a\*b\* system, emulsifying capacity, susceptibility to enzymatic hydrolysis with  $\alpha$ -amylase. On the basis of the results it was determined that OSA starches modified using iron ions were characterised by similar values of SW and WBC, irrespective of OSA concentrations. Incorporation of iron ions to the OSA starches resulted in a decrease in brightness ( $L^*$ ), furthermore it caused an increase in the values of parameters  $a^*$  and  $b^*$ . Modification using iron ions resulted in a decrease of susceptibility to enzymatic hydrolysis as compared to non-modified OSA starches. Emulsion prepared with 6% OSA modified with iron was the most stable during storage among the analysed starch preparations.

**Keywords:** OSA starch, enrichment, iron ions, physicochemical properties

### INTRODUCTION

Starch is one of carbohydrate polymers widely used in food products. The use of native starch is limited due to its low stability under different conditions, such as very low or high temperature, low pH or shear rate commonly applied to processed food. In order to improve starch properties, it is subjected to physical and chemical modifications. Esterification using octenyl succinic acid anhydride (OSA) is one of the methods of starch chemical modification. With the incorporation of hydrophobic alkenyl groups into a hydrophilic starch molecule, the modified starch obtains surface active properties which are useful in stabilising oil/water emulsions. As a result, aqueous solutions of OSA starch have been used to stabilise flavour concentrates in

beverages and oil in salad dressings (Shorgen *et al.* 2000). Furthermore, it was found that OSA starch decreases the extent of starch digestion. This indicates that OSA starch appears to be a resistant starch (Song *et al.* 2006).

Mineral elements constitute a group of chemical compounds indispensable for proper body functioning. In a human body they serve building functions, control metabolic processes, ionic balance and cell pressure, and sustain neuromuscular excitability. Nowadays, due to contemporary lifestyle and changes in human diet, demands for mineral elements supplied with food are observed to increase (Pietrzyk *et al.* 2013). The mineral elements most frequently lacking in human diets are Fe, Zn and I, although other elements, such as Ca, Mg, Cu and Se, can be deficient in the diets of some populations. It is estimated that, of the world's 6 billion people, 60-80% are Fe deficient. Traditional strategies to deliver these minerals to susceptible populations have relied on supplementation or food fortification (White and Broadley 2005). Thereby, it is feasible to apply OSA starch (widely used as a food additive) as a carrier of micro- and macroelements.

The presence of hydroxy and octenyl succinate groups in OSA starch chains allows the binding of metal ions to them. Nevertheless, the presence of mineral elements incorporated to OSA starches not only increases their nutritive value but also affects their physicochemical properties. Hence, the aim of this work was to evaluate the effect of iron ion on the physicochemical properties of OSA starch.

## MATERIAL

Native maize starch used for the study was purchased from Młyn Gospodarczy Paweł Bogutyn (Radzyń Podlaski, Poland). High-purity octenyl succinic acid anhydride (OSA) was supplied from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The other chemicals used in the study were of analytical grade.

OSA starch samples were prepared according to Hui *et al.* (2009) procedure. Potato starch (300 g, d.w.) was suspended in distilled water with agitation to obtain 35% (w/w) starch concentration. The pH of the suspension was adjusted to 8.0 with a pH-meter by dripping in a 3% NaOH solution. OSA solution, in concentrations of 3%, 6%, 9% (calculated on d.w. of starch) five-fold diluted with absolute alcohol, was added slowly during 1 h. The reaction was continued for 3.5 h with sample agitation. After required time, the pH was adjusted to 6.5 with 3% HCl solution, the mixture was centrifuged, washed twice with distilled water and 70% aqueous alcohol. Then the solid was oven-dried at 45°C for 24 h, then passed through mechanical sieve with mesh diameter of 200 µm. To obtain a blank sample, the same process was carried out without any addition of OSA to the starch solution (0% OSA).

OSA starches were examined for the degree of substitution (DS). DS is the average number of hydroxyl groups substituted per glucose unit. The DS of OSA starch was determined by titration (Hui *et al.* 2009). An OSA starch sample (5 g, d.w.) was accurately weighed and dispersed by stirring for 30 min in 25 cm<sup>3</sup> of 2.5 M HCl isopropyl alcohol solution. 100 cm<sup>3</sup> of 90% (v/v) aqueous isopropyl alcohol solution were added and the slurry was stirred for an additional 10 min. The suspension was filtered through a glass filter and the residue was washed with 90% aqueous isopropyl alcohol solution until no Cl<sup>-</sup> could be detected any longer. The starch was re-dispersed in 300 cm<sup>3</sup> of distilled water, and then the dispersion was cooked in a boiling water-bath for 20 min. The starch solution was titrated with 0.1 M standard NaOH solution, using phenolphthalein as an indicator. Simultaneously, native starch was also titrated as a blank sample. The DS was calculated by the following equation:  $DS = \frac{0.162(A \cdot M)/W}{1 - [0.210(A \cdot M)/W]}$ ,

where: *A* is the titration volume of NaOH solution (cm<sup>3</sup>), *M* is the molarity of NaOH solution, and *W* is the dry weight (g) of the starch.

Afterwards, starches with different OSA concentration were modified by reaction with iron ions. 100 g of starch was mixed with 250 cm<sup>3</sup> of distilled water for 5 min and then the resulting dispersion was strained through a filter funnel. 200 cm<sup>3</sup> of a mixture of 1% (w/w) FeSO<sub>4</sub> was added to the starch and the whole was stirring for 5 min. The resulting product was strained through filter funnel and 200 cm<sup>3</sup> of the mixture of 1% FeSO<sub>4</sub> was added to the retentate and the sample was stirred for 5 min. The action was repeated one more time. The starch was rinsed with distilled water until sulphurions were absent (using 0.1 M BaCl<sub>2</sub> solution), and then it was filtered, air dried and sieved.

## METHODS

OSA starches modified with iron ions were examined for iron content according to the Polish Standard PN-91R-04014. The starch was mineralised in a mixture of nitric and sulphuric acids at temperature of 250°C (Wet Digester, Büchi, Switzerland). The quantitative analysis was performed by atomic absorption spectrometry (Avanta Sigma, GBC, Australia) using acetylene/air flame.

Water binding capacity and solubility in water at temperature of 50 and 60°C were determined by the modified method of Leach (Richter *et al.* 1968). Starch suspension in concentration of 1.25% (w/v) was heated during 30 min at temperature of 50 and 60°C and after that the starch paste produced was recovered by mild centrifugation (2500 x g, x 15 min). Water binding capacity was calculated on dry weight of starch basis and solubility was based on soluble starch.

Colour parameters of the samples were determined in the CIE L\*a\*b\* system (measuring geometry d/8°, gap 10 mm, illuminant D65, observer 10°) using a Colour i5 spectrophotometer (X-Rite, USA). Total colour difference ( $\Delta E$ ) between 0% OSA (blank sample) and modified starches was calculated according to the equation:

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

where: L\* – lightness (from 0 = black to 100 = white), a\* (+a = redness; -a = greenness), b\* (+b = yellowness; -b = blueness).

The starch susceptibility to enzymatic hydrolysis was determined using  $\alpha$ -amylase type VI-B, from porcine pancreas, activity 28 u/mg solid (Sigma-Aldrich, USA) according to the method of Hung and Marita (2005) with some modification. To 40 mg of starch were added 38 cm<sup>3</sup> of water and 2 cm<sup>3</sup> solution of enzyme (10 mg of  $\alpha$ -amylase in 10 cm<sup>3</sup> 0.05 M NaCl). The enzymatic hydrolysis of starch was carried out at temperature of 37°C. Maltose content after hydrolysis was measured on the basis of colour reaction with 3,5-dinitrosalicylic acid after 20, 40, 60, 80, 100 and 120 min using a V-630 spectrophotometer (Jasco, Germany). The extent of starch hydrolysis was presented as the percentage content of maltose in starch.

Emulsifying capacity of the emulsions was also investigated. Starch (1.0 g, dry weight) was suspended in distilled water (100 cm<sup>3</sup>) and heated in a boiling water bath for 15 min. After cooling down to room temperature, the starch paste was mixed with 50 cm<sup>3</sup> of soybean oil using high shear dispersing emulsifier Ultra-Turrax T-25 (Ika-Werke, Germany) at 10 000 rpm for 2 min. Twenty five millilitres of the emulsion was poured into a graduated test tube. Emulsifying capacity after 10 days of storage was characterised by creaming index (CI, %) which was calculated according to the equation:

$$CI = \frac{H_s}{H_t} \cdot 100\%$$

where:  $H_s$  is the height of the serum layer and  $H_t$  is the total height of the emulsion (Li *et al.* 2013).

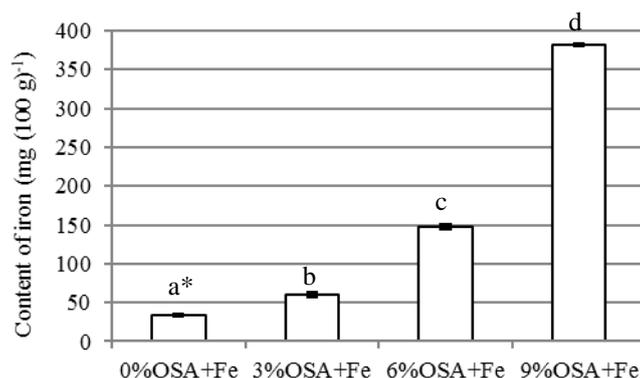
The droplet images of the emulsions were captured under optical microscope Eclipse E200 (Nikon, Japan) at room temperature. A small drop of emulsion was placed onto the microscope slide and carefully covered. Photomicrographs were taken using a digital camera (Digital Ixus 100 IS, Canon, Japan).

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

## RESULTS AND DISCUSSION

Maize starch modified with different levels of OSA was subjected to DS analysis. DS values of 3%, 6%, 9% OSA starches were 0.007, 0.017, 0.025, respectively. A similar trend in the reactivity of OSA with starches was observed by other authors (Bao *et al.* 2003, Hui *et al.* 2009). The efficiency and rate of the chemical modification process depend on the reagent type, botanical origin, the size and structure of starch granules (Huber and Be Miller 2001).

Modification using iron ions was effective, as the amount of the metal ions contained in the OSA starches increased after the modification (Fig. 1). Content of iron in all tested OSA starches increased with an increase of the degree of substitution. There was a high positive correlation between the DS values and the contents of iron ( $r = 0.95$ ), and this result suggests that the presence of OS groups allows binding of the metal ions to polysaccharide chains, thus forming metal-starch complexes. 9%OSA+Fe starch was the most abounding in metal ions, as their amount was almost three-fold higher than in 6%OSA+Fe. Similar results was obtained in our previous study (Królikowska *et al.* 2013), where it was demonstrated that enrichment of OSA potato starch with calcium ions caused an increase of calcium content in starch. Pietrzyk *et al.* (2013) also noted an increase in iron content in oxidised starches of different botanical origin after their modification using iron ions.



**Fig. 1.** Content of iron in tested OSA starch preparations after modification with iron ions. Within columns, values with the same letter (a, b, c, d) do not differ significantly at  $\alpha = 0.05$ . The vertical sections on the bars represent standard deviations.

The water-binding capacity and starch solubility in water at temperatures of 50 and 60°C are presented in Table 1. Values of WBC and SW of OSA starches non-modified with iron increased with an increase in the concentration of % OSA used for modification, at both temperatures used in the study. Special attention should be paid to the fact that 9%OSA starch at 50°C has a 15 times greater SW and at 60°C 6 times greater WBC than 0%OSA. The increase in the SW and WBC may be due to weakening of intermolecular hydrogen bond due to the introduction of OS groups (Perez *et al.* 1993). A similar phenomenon was observed by Bhosale and Singhal (2007). Thanks to this property it is possible to use OSA starches as a thickening agent in processes where starch must form a gel at lower temperatures than usual. Starches 6%OSA and 9%OSA modified using iron were characterised by lower values of SW and WBC as compared to respectively non-OSA enriched starches. Furthermore, OSA preparations modified with iron ions did not differ significantly from each other in the values of parameters SW and WBC (except 0%OSA+Fe). The WBC of starch is determined, most of all, by amylopectin, whereas its solubility– by amylase (Tester and Morrison1990).

**Table 1.** Water binding capacity and solubility in water of tested OSA starch preparations before and after modification with iron ions

Starch	Solubility in water(%)		Water binding capacity (g (g d.w.) <sup>-1</sup> )	
	50°C	60°C	50°C	60°C
0% OSA	0.27±0.02 <sup>c</sup>	1.74±0.07 <sup>a</sup>	1.73±0.13 <sup>a</sup>	1.98±0.01 <sup>b</sup>
3% OSA	0.74±0.08 <sup>d</sup>	1.03±0.18 <sup>a</sup>	2.32±0.01 <sup>a</sup>	3.15±0.06 <sup>c</sup>
6% OSA	2.10±0.06 <sup>a</sup>	3.93±0.28 <sup>c</sup>	4.21±0.13 <sup>c</sup>	6.79±0.62 <sup>d</sup>
9% OSA	4.13±0.26 <sup>e</sup>	5.62±0.45 <sup>d</sup>	6.32±0.45 <sup>d</sup>	11.28±0.33 <sup>e</sup>
0% OSA+Fe	1.97±0.04 <sup>a</sup>	2.09±0.05 <sup>e</sup>	3.01±0.07 <sup>b</sup>	3.78±0.18 <sup>a</sup>
3% OSA+Fe	0.99±0.12 <sup>b</sup>	1.16±0.11 <sup>a,b</sup>	3.06±0.33 <sup>b</sup>	3.52±0.23 <sup>a</sup>
6% OSA+Fe	1.19±0.06 <sup>b</sup>	1.60±0.08 <sup>b</sup>	3.66±0.04 <sup>b</sup>	4.01±0.48 <sup>a</sup>
9% OSA+Fe	1.15±0.04 <sup>b</sup>	1.47±0.15 <sup>b</sup>	3.71±0.35 <sup>b</sup>	4.13±0.22 <sup>a</sup>

Within columns, values with the same letter (a, b, c, d) do not differ significantly at  $\alpha = 0.05$ .

Colour parameters of modified starch preparations are presented in Table 2. All OSA starches (non-modified with iron ions) did not differ significantly in the values of lightness (L\*) and a\* parameter. They were characterised by negative values of parameter a\* which indicates the predominance of the green component in the colour of starch, and positive values of parameter b\* which demonstrate

a prevalence of the yellow component. Modification using iron ions caused a significant decrease in the lightness of the OSA starches, as well as an increase in the values of parameters  $a^*$  and  $b^*$ , which means greater participation of red and yellow components in the starch. The yellow colour is typical of Fe(III) ion complexes, which may suggest the presence of trivalent iron in modified starches. Special attention should be paid to the fact that 9%OSA starch was characterised by 5 times greater value of  $a^*$  and 3 times greater value of  $b^*$  as compared to 0%+Fe starch. The values of correlation coefficient between iron content (Fig.1) and colour parameters  $L^*$ ,  $a^*$ ,  $b^*$  were  $r_{L^*} = -0.90$ ,  $r_{a^*} = 0.90$ ,  $r_{b^*} = -0.97$ , respectively. These results are consisted with those of Śmigielska *et al.* (2005) for oxidised starches incorporated with iron. Those authors also found out a decrease in  $L^*$  and an increase in  $a^*$  and  $b^*$  parameters of modified starches, which suggested that the colour parameters depend on the quantity of incorporated mineral. The values of  $\Delta E$  parameter (total colour difference) for all OSA starches modified with iron ions were above 2, which indicates noticeable for untrained eyes changes in colour. Other OSA starches, non-modified with iron, were characterised by  $\Delta E$  values below 2, which indicates a lack of differences in the colour of these starches as compared to blank sample (Francis and Clydesdale 1975).

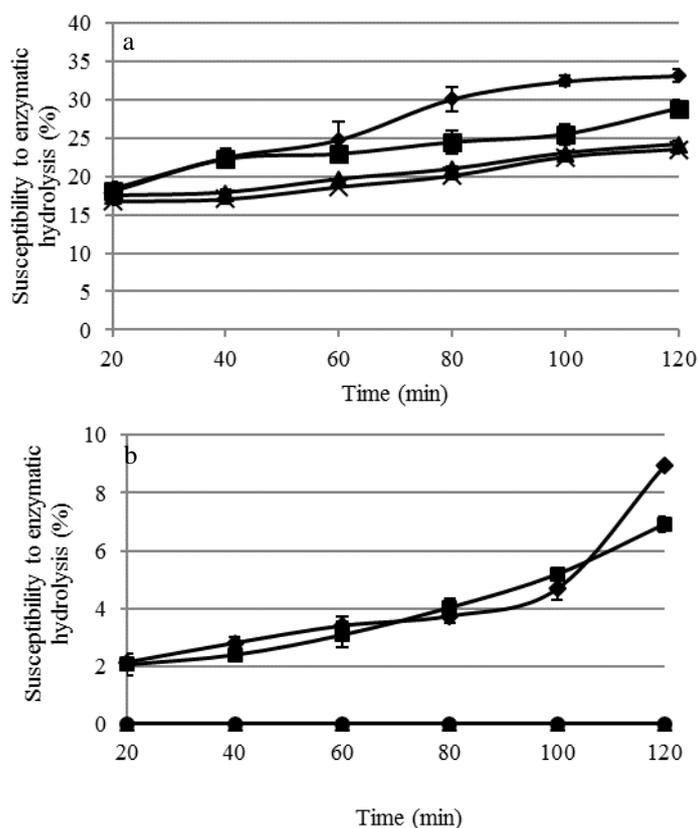
**Table 2.** Colour parameters of tested OSA starch preparations before and after modification with iron ions

Starch	$L^*$	$a^*$	$b^*$	$\Delta E$
0%OSA	93.96±0.14 <sup>a</sup>	-1.02±0.05 <sup>a</sup>	4.45±0.10 <sup>b</sup>	-
3%OSA	94.13±0.20 <sup>a</sup>	-1.05±0.04 <sup>a</sup>	4.26±0.10 <sup>ab</sup>	0.26
6%OSA	93.78±0.14 <sup>a</sup>	-1.13±0.04 <sup>a</sup>	4.04±0.05 <sup>ab</sup>	0.46
9%OSA	93.94±0.24 <sup>a</sup>	-1.09±0.02 <sup>a</sup>	3.94±0.10 <sup>a</sup>	0.52
0%OSA+Fe	91.79±0.06 <sup>b</sup>	-0.54±0.01 <sup>b</sup>	5.91±0.08 <sup>c</sup>	2.67
3%OSA+Fe	89.95±0.06 <sup>c</sup>	0.32±0.03 <sup>c</sup>	9.99±0.21 <sup>d</sup>	6.97
6%OSA+Fe	85.83±0.07 <sup>d</sup>	1.70±0.09 <sup>d</sup>	16.03±0.14 <sup>e</sup>	14.41
9%OSA+Fe	83.91±0.05 <sup>e</sup>	2.47±0.09 <sup>e</sup>	18.03±0.18 <sup>f</sup>	17.26

Within columns, values with the same letter (a, b, c, d, e, f) do not differ significantly at  $\alpha = 0.05$ .

Figures 2a and 2b show the susceptibility of the OSA-starches (non-modified and modified with iron) to enzymatic hydrolysis with  $\alpha$ -amylase. It is widely known that starch granules are less susceptible to amylase hydrolysis than gelatinised starch.  $\alpha$ -amylase is an endoamylase which is capable of hydrolysing internal  $\alpha$ -1,4-glycosidic bonds in the long chains of starch. It cannot hydrolyse  $\alpha$ -1,6-glycosidic bonds, however, it can pass these bonds and continue to hydrolyse  $\alpha$ -1,4-

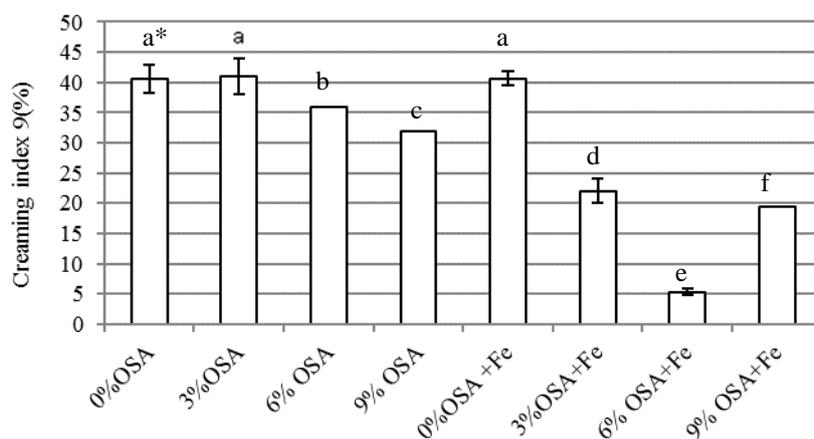
glycosidic bonds. Thanks to this property  $\alpha$ -amylase hydrolyses starch granules faster than other enzymes (Ma *et al.* 2006). Susceptibility to enzymatic hydrolysis of OSA-starches decreased with an increase in the concentration of OSA used for modification. The greatest susceptibility to enzymatic hydrolysis was observed for the 0% OSA starch, whereas the lowest for 6% OSA and 9% OSA starches. Viswana than also (1999) showed that resistance to enzyme hydrolysis was proportional to the degree of substitution of OSA starches. The applied modification with iron ions caused a decrease in susceptibility to hydrolysis with  $\alpha$ -amylase as compared to non-modified with iron OSA preparations. OSA+Fe starches were characterised by a greater resistance to degradation as compared to non-modified OSA starches and this resistance depends on the amount of incorporated mineral.



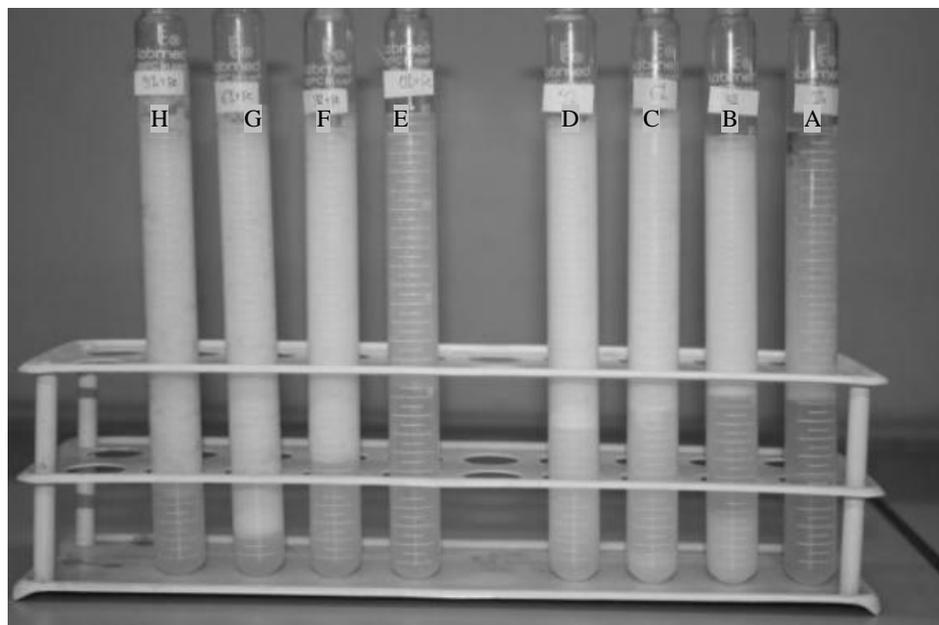
**Fig. 2.** Susceptibility to enzymatic hydrolysis of (a) OSA starches and (b) OSA+Fe starches, (♦) 0%, (■) 3%, (▲) 6%, (●) 9%. The vertical sections on the bars represent standard deviations.

The enzyme activity was highly disturbed by the presence of iron. This conclusion is corroborated by findings of Hemalatha and Venkastestan (2011) who demonstrated that amylase activity decreases with increasing iron concentration. Moreover, those authors claimed that iron forms complexes with carbohydrate compounds like maltose, which caused a reduction in enzyme activity.

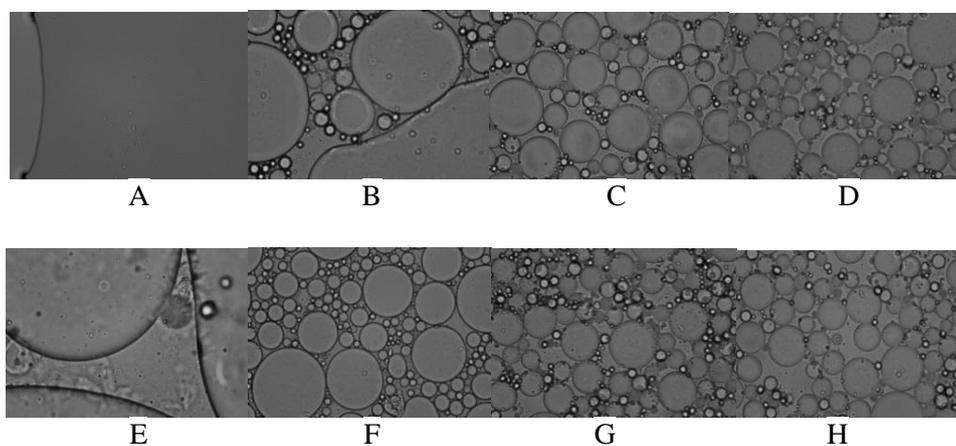
Emulsification capacity is a very important factor for the application of starch in the food industry. The results of the effect of modification with OSA and iron on the creaming index and the visual appearance of O/W emulsions stored for 10 days are presented in Figures 3 and 4. For all tested preparations, increasing OSA concentration and iron content led to a decrease in the values of creaming index. The emulsions formed with 0% OSA, 3% OSA and 0% OSA+Fe developed similar amounts of serum. However, the emulsions prepared with 0% OSA and 0% OSA+Fe starches showed the appearance of very large oil globules and separated water (Fig. 4). The greatest storage stability was observed in the case of 9% OSA starch, whereas among the starches modified with iron, the best stability was characteristic of 6% OSA+Fe starch. In general, emulsions formed by OSA starches incorporated with iron appeared to be more stable during storage. OSA starches are recommended as a stabiliser for different food emulsions (Bao *et al.* 2003, Li *et al.* 2013, Tesch *et al.* 2001). OSA starches can act as steric stabilisers due to the size of the molecules relative to the number of charged groups (Sweedman *et al.* 2013). Moreover, the mechanism of steric stabilisation of oil droplets by OSA starch colloids was also observed by Song *et al.* (2013).



**Fig. 3.** Creaming index of tested OSA starches before and after modification with iron ions  
\*Within columns, values with the same letter (a, b, c, d, e, f) do not differ significantly at  $\alpha = 0.05$ .  
The vertical sections on the bars represent standard deviations.



**Fig. 4.** Photographs of emulsions prepared with tested starch preparations and soybean oil: (A) 0% OSA, (B) 3% OSA, (C) 6% OSA, (D) 9% OSA, (E) 0% OSA+Fe, (F) 3% OSA+Fe, (G) 6% OSA+Fe, (H) 9% OSA+Fe



**Fig. 5.** Optical micrographs of the emulsions formed by tested starch preparations and soybean oil: (A) 0% OSA, (B) 3% OSA, (C) 6% OSA, (D) 9% OSA, (E) 0% OSA+Fe, (F) 3% OSA+Fe, (G) 6% OSA+Fe, (H) 9% OSA+Fe

Optical micrographs of the emulsions after storing for 10 days at room temperature are shown in Figures 5A-H. The micrographs suggest that starches with different OSA concentrations could act as effective emulsifiers, because the droplet sizes of emulsions were smaller as compared to 0% OSA starch. The emulsion with 0% OSA contained large oil droplets. Previous studies conducted by Song *et al.* (2013) indicate that with increasing OSA concentration used for modification the changes in droplet sizes of the emulsion decrease. These data are in accordance with the results obtained in our study.

### CONCLUSIONS

1. On the basis of the results it was determined that incorporation of iron ions into starch granules did not affect the water binding capacity and solubility in water of OSA+Fe starch preparations.
2. The analysis of colour parameters indicated that incorporation of iron ions to the OSA starches resulted in a decrease in brightness ( $L^*$ ), as well as caused an increase in the values of the parameters  $a^*$  and  $b^*$ .
3. Modification using iron ions caused a decrease in susceptibility to enzymatic hydrolysis using  $\alpha$ -amylase of tested OSA starches.
4. Starch enrichment with iron ions caused an increase in emulsion stability during storage, and the emulsion prepared with 6% OSA+Fe starch was the most stable.

### ACKNOWLEDGEMENT

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## WPLYW JONÓW ŻELAZA NA WYBRANE WŁAŚCIWOŚCI FIZYKOCHEMICZNE OKTENYLOBURSZTYNIANÓW SKROBIOWYCH

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**Streszczenie.** Naturalną skrobię kukurydzianą estryfikowano z wykorzystaniem bezwodnika kwasu oktenylobursztynowego (OSA) przy czterech stężeniach: 0%, 3%, 6%, 9% i następnie wzbogacono w jony żelaza. Celem pracy było określenie wpływu jonów żelaza na właściwości fizykochemiczne skrobi estryfikowanej OSA. Skrobie modyfikowane poddano następującym analizom: oznaczeniu zawartości żelaza, zdolności wiązania wody i rozpuszczalności w wodzie w temperaturach 50 i 60°C, pomiarowi barwy w systemie CIE  $L^*a^*b^*$ , zdolności emulgowania oraz podatności na hydrolizę enzymatyczną z wykorzystaniem  $\alpha$ -amylazy. Na podstawie uzyskanych wyników stwierdzono, że skrobie OSA modyfikowane jonami żelaza nie różniły się pod względem wartości zdolności wiązania wody i rozpuszczalności w wodzie niezależnie od stężenia OSA. Wzbogacenie skrobi OSA w jony żelaza spowodowało zmniejszenie jasności ( $L^*$ ) skrobi oraz wzrost wartości parametrów  $a^*$  i  $b^*$ . Modyfikacja z wykorzystaniem jonów żelaza spowodowała zmniejszenie podatności na hydrolizę enzymatyczną badanych skrobi w porównaniu do ich niemodyfikowanych odpowiedników. Emulsja przygotowana ze skrobi estryfikowanej OSA przy stężeniu 6% i modyfikowanej jonami żelaza spośród badanych preparatów skrobiowych była najbardziej stabilna podczas przechowywania.

**Słowa kluczowe:** skrobia OSA, wzbogacanie, jony żelaza, właściwości fizykochemiczne