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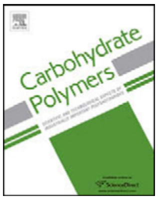

Physicochemical characterisation of enzymatically hydrolysed derivatives of acetylated starch

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ABSTRACT

Potato starch modified to different degrees by substitution with acetyl groups was the subject of this study undertaken to determine the influence of conditions of enzymatic hydrolysis on the surface-active properties of hydrolysates of acetylated starch. The effect of acetylation of starch preparation on its susceptibility to enzymatic hydrolysis in the membrane reactor was also considered. All hydrolysates of acetylated starch samples investigated were found to bring a decrease in the surface/interfacial tension, both at the air/water and the toluene/water interfaces. For binary hydrolysate–surfactant systems, the surface mole fractions in the mixed adsorbed monolayer at the air/water interface were estimated. For mixed systems, the synergism in reducing the surface tension at the air/water interface was observed. The experimentally obtained dynamic surface tension data for the aqueous solution of acetylated starch hydrolysates were used to estimate the diffusion coefficients. Particle size distributions of the hydrolysates formed in the aqueous solutions were compared to those of commercial maltodextrin.

1. Introduction

Chemical modification of starch for food application is realised by introduction of modifying groups into the macromolecules of starch through oxidation, esterification or etherification reactions (Murray, Durga, Yusoff, & Stoyanov, 2011; Wurzburg, 1986; Yusoff & Murray, 2011). The acetylation process seems to be especially important, because it permits incorporation of relatively hydrophobic modifying groups into hydrophilic starch macromolecules. Food grade modified starches containing acetyl groups, i.e. acetylated starch E 1420, acetylated distarch adipate E 1422, and acetylated distarch phosphate E 1414, are recommended for manufacturing food products of emulsion type because of their significant surface activity. Starches modified by acetylation are commonly used and applied in food industry as stabilizers, thickeners and binding agents (Chi et al., 2008; Elomaa et al., 2004; Raina, Singh, Bawa, & Saxena, 2006; Yadav, Mahadevamma, Tharanathan, & Ramteke, 2007). Moreover it was proved that the products of hydrolysis of oxidized starch or starch modified with octenylsuccinic anhydride reveal higher surface activity than their high molecular mass counterparts (Kędziora, Le Thanh, Lewandowicz, & Prochaska, 2006; Prochaska, Kędziora, Le Thanh, & Lewandowicz, 2007). Additional hydrolysis of acetylated starches might significantly broaden the area of functional food additives and starch products application (Prochaska et al., 2007a). However, before implementation of a new product (food additive) onto the market, it is necessary to fully characterize this substance in terms of its structure, physicochemical properties and functionality.

Application of a continuous recycle enzymatic membrane reactor (CRMR), being a combination of the enzymatic reactor and the membrane separation, is often proposed for the hydrolysis of starch, especially for the manufacturing of low molecular weight products such as glucose, maltose, starch syrups or cyclodextrines (Sakinah et al., 2008; Sarbatly & England, 2004; Sims & Cheryan, 1992a; Sims & Cheryan, 1992b). CRMR offers such advantages as high enzyme loads, prolonged enzyme activity, high flow rates, costs, reduction in energy requirements and in waste products by the use of recycling, easy operation and control, straightforward scale-up to large systems and high yields of pure material (Kędziora et al., 2006; Sakinah et al., 2008). As a raw material for hydrolysis, commercial starches obtained from corn, tapioca and potato (Grześkowiak-Przywecka & Słomińska, 2007; Paolucci-Jeanjean, Belleville, Rios, & Zakhia, 2000; Sarbatly & England, 2004; Sims & Cheryan, 1992b) have been proposed. These stocks reveal different performance in CRMR (mainly due to different viscosity of their pastes), but provide the same products. The use of chemically modified starch as a raw material for hydrolysis in CRMR opens the possibility of manufacturing new products with useful

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properties for food technology. However, the presence of modifying groups may affect the susceptibility of starch to enzymatic hydrolysis (Le Thanh et al., 2007). The data regarding susceptibility of chemically modified starches are relatively rare and often contradictory. Enzyme action has been reported to be inhibited by the presence of phosphate groups in the modified starch molecules and the extent of inhibition increases with increasing degree of phosphate substitution (Sitohy & Ramadan, 2001). On the other hand, Raben, Andersen, Karberg, Holst, & Astrup (1997) claim that the addition of acetylated potato starch improved the glycemic, insulinemic and satiating properties in a meal from an in vivo test. It was also reported that the susceptibility to enzymatic hydrolysis depended on degree of substitution of starches, especially the cross-linked starches (Hoover & Sosulski, 1986; Hoover, Hannouz, & Sosulski, 1988). The discrepancies described above suggest the necessity of more extensive studies on enzymatic hydrolysis of chemically modified starches.

The subject of this study was potato starch modified by different degrees of substitution with acetyl groups. The aim of the work was to determine the influence of conditions of the enzymatic hydrolysis process in the recycle membrane reactor on the surface-active properties of hydrolysates of acetylated starch obtained. The influence of the transmembrane pressure applied for separation of the reaction mixture on the efficiency of hydrolysis as well as on the capability of hydrolysates obtained of reducing the surface tension at the air/water or oil/water interface was examined. The effect of the degree of substitution of acetylated starch on its susceptibility to enzymatic hydrolysis in the membrane reactor was also evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Acetylation of starch

Commercial native potato starch "Superior Standard" produced by WPPZ Luboń (Poland) was used as a raw material in the esterification process. Acetylation was carried out in suspension using acetic anhydride with dilute sodium hydroxide as a catalyst. The starch suspension was neutralized, filtered and washed in order to remove by-products. The products obtained, with various degrees of substitution, were dried in air at room temperature. The content of acetyl group was determined by acidimetric titration of acetic acid released after hydrolysis, according to the procedure recommended by JECFA (Joint FAO/WHO Expert Committee on Food Additives) (FAO, 1997; Sodhi & Singh, 2005).

2.1.2. Hydrolysis in a membrane reactor

The acetylated starch samples of different degrees of substitution (DS), viz. 0.02 and 0.1 were subjected to the hydrolysis process with BAN 480L as a hydrolytic enzyme preparation. BAN 480L, kindly provided by Novozymes (Denmark), is a bacterial α-amylase (EC 3.2.1.1) produced by selected strains of *Bacillus amyloliquefaciens*. The optimum activity of the enzyme preparation BAN 480L is achieved at temperatures from the range 60 to 70 °C and pH 5.5 to 7.0.

The hydrolysis process was performed in a continuous recycle membrane reactor (CRMR) consisting of a 5 L reaction vessel BioFlo III (New Brunswick Scientific Co., Inc., USA), a tubular, ceramic membrane (Tami Industries, France) and a rotary lobe pump (Johnson, USA). A scheme of the reactor used is shown in our previous work (Kędziora et al., 2006). The membrane was characterised by the following parameters: a length of 1.2 m, external diameter of 25 mm, eight channels, each 6 mm in diameter and with surface area of 0.2 m^2, and molecular weight cut off of 50 kDa. Each hydrolysis was performed using 5% (w/w) solutions of the starch prepared using gelatinisation and sterilisation. To start the hydrolysis process, the reactor was filled with 7 L of the starch solution of pH 6.0 (optimum pH of enzyme activity) and then 0.1 ml of enzyme was added. Next, the reaction mixture, at a temperature of 60 °C, was continuously pumped to the membrane module and the retentate flux was recycled back to the reaction vessel. The hydrolysis was carried out in the time frame of 100 min. The constant level of volume of the reaction mixture in reaction vessel was controlled automatically, and the loss of the reaction mixture volume was supplemented with starch solution during the first 60 min of the process, afterwards with water. The permeate as well as retentate fractions were collected and dried in a Mobile Miner^{TM} 2000 (Niro A/s) spray dryer.

During the hydrolysis process of each of acetylated derivatives, the UF separation was performed at three different transmembrane pressures (low 0.05 MPa (called later *L*), medium 0.1 MPa (*M*) and high 0.15 MPa (*H*)). As a final result of enzymatic hydrolysis, six hydrolysates of acetylated starch with two different DS (0.02 and 0.1) were obtained for subsequent analyses.

Because of the hygroscopicity of the hydrolysates investigated, the moisture content of the individual fractions described in Table 1 was taken into consideration when the aqueous solutions were prepared.

The dry substance content of the permeate and retentate, as well as the moisture content of the products obtained by spray drying were determined by an oven-drying method (PN-EN ISO 1666:2000; PN-78/A-74701).

The percent of the total solids that have been converted to reducing sugars, and amounts of reducing groups (dextrose equivalent – DE) were determined by the modified School-Rogenbogen method (PN-78/A-74701).

2.2. Methods

2.2.1. Surface and interfacial tension measurements

The surface-active properties of all hydrolysates obtained were studied by the equilibrium and dynamic surface/interfacial tension experiments performed in two systems, namely oil/water and air/water.

The equilibrium surface/interfacial tension of the acetylated starch samples and the products of enzymatic hydrolysis (i.e. the dried solutes of permeates) was measured by the du Noüy ring method with a KRÜSS K12 tensiometer (Germany) (Rusanov & Prokhorov, 1996). The dynamic measurements were performed by the drop shape method with a TRACKER I.C. Concept instrument (France) (Rusanov & Prokhorov, 1996). The standard deviation of the surface/interfacial tension measurements was 0.05 mN/m. All measurements were carried out at 21 ± 0.1 °C. Twice distilled water with the conductivity of 3 μS was used as the aqueous phase. The toluene used as the organic phase for interfacial tension measurements was obtained from Aldrich Company. It is obvious that the toluene cannot be used as a food additive, however it was applied in our investigations as the model organic phase (Kędziora et al., 2006; Prochaska et al., 2007a; Prochaska, Kędziora, Le Thanh, & Lewandowicz, 2007b; Prochaska, Konował, Sulej-Chojnacka, & Lewandowicz, 2009).

2.2.2. Micelle size distribution

The micelle size distribution (MSD) in 3% aqueous solutions of the starch derivatives investigated was measured using the dynamic light scattering method (DLS) (Berne & Pecora, 1976) on a Zeta-Sizer Nano ZS apparatus (Malvern Instruments Ltd., Great Britain). In the instrument, the size of the particles is measured by observing the scattering of laser light from these particles, determining the rate of diffusion and deriving the size from this, using

Table 1
Adsorption parameters for hydrolysates in the air/water and oil/water systems.

Parameter		$\Gamma^\infty \times 10^6$	$A_{\min}$	$-\Delta G_{\text{ads}}$
Unit		mol/m^2	nm^2	kJ/mol
Air/water system	$L_{0.10}$	3.07	0.54	11.68
	$M_{0.10}$	2.27	0.73	16.86
	$H_{0.10}$	1.05	1.58	24.43
Oil/water system	$L_{0.10}$	0.81	2.06	13.56
	$M_{0.10}$	0.59	2.83	14.51
	$H_{0.10}$	0.42	3.99	18.54

the Stokes–Einstein relationship. The measured data in a DLS experiment is the intensity autocorrelation curve.

2.2.3. *Calculations*

Experimentally obtained surface/interfacial tension isotherms were approximated by the Szyszkowski isotherm (1) (Chattoraj & Birdi, 1984):

$$\gamma_i = \gamma_0 \left[1 - B_{SZ} \ln\left(\frac{c}{A_{SZ}} + 1\right)\right] \quad (1)$$

where γ_0 is the surface/interfacial tension for the distilled water and A_{SZ} and B_{SZ} are the coefficients of Szyszkowski isotherm.

The Szyszkowski coefficients (A_{SZ} and B_{SZ}) were used to estimate the surface excess (surface concentration) at the saturated interface (Γ^∞) (Eq. (2)) and the free energy of adsorption (ΔG_{ads}) (Eq. (3)) (Kędziora et al., 2006). The used equations are presented below:

$$\Gamma^\infty = \frac{\gamma_0 B_{SZ}}{RT} \quad (2)$$

$$\Delta G_{ads} = -RT \ln \quad A_{SZ} \quad (3)$$

where R is the gas constant and T is the temperature.

All adsorption parameters were estimated assuming that an anhydroglucose unit is the equivalent of one mole of modified starch or its hydrolysis products (Kędziora et al., 2006; Lundqvis, Eliasson, & Olofsson, 2002).

The surface activity of the relevant substance was also characterised by its adsorption efficiency, defined as the minimum bulk phase concentration ($-\log c_1 = pC_1$) required to produce a saturated interface (Osseo-Asare, 1984).

The experimentally obtained dynamic surface tension data for the aqueous solution of acetylated starch hydrolysates were used to estimate the diffusion coefficients. Dynamic surface tension curves were analyzed using the asymptotic solution at long times of the classical Ward and Tordai equation (Fainerman, Makievski, & Miller, 1994), which describes the adsorption process of the surfactant in an aqueous solution. When it is assumed that the adsorption process is diffusion controlled and moreover, when the adsorption process is near the equilibrium, the approximation can be written as:

$$\gamma_{t\to 8} = \gamma_{eq} + \left[\frac{nRT\Gamma_{eq}^2}{c}\right]\sqrt{\frac{\pi}{4Dt}} \quad (4)$$

The parameters c, Γ, and D represent the bulk concentration, equilibrium surface excess and monomer diffusion coefficient of the surfactant, $n = 1$ for neutral molecules, and $n = 2$ for ions, T is absolute temperature (Álvarez Gómez, Pizones Ruíz Henestrosa, Carrera Sánchez, & Rodríguez Patino, 2008; Calvo, Bravo, Amigo, & Gracia-Fadrique, 2009; Deme, Rosilio, & Baszkin, 1995; He et al., 2008; Liggieri, Ravera, & Passerone, 1995; Prochaska, Walczak, & Staszak, 2002; Staszak & Prochaska, 2006).

For binary hydrolysate–surfactant systems, the surface mole fractions of the adsorbed compounds in the mixed adsorbed monolayer at the air/water interface were analyzed by the model developed by Rosen et al. (Gu & Rosen, 1989; Rosen, 1986; Rosen & Murphy, 1989) and estimated according to Eq. (2)

$$\frac{X_1^2 \ln(c_{1,t}/(c^0_{1,t}X_1))}{(1-X_1)^2 \ln(c_{2,t}/(c^0_{2,t}(1-X_1)))} = 1 \quad (5)$$

where X_1 is the mole fraction of the hydrolysate in the mixed adsorbed monolayer, α is the molar fraction of hydrolysate in the bulk solution, $c_{1,t} = \alpha_{ct}$ are the total molar concentrations of the hydrolysate and the surfactant, respectively, in their mixture needed to produce a given interfacial tension (reduction). The symbols $c^0_{1,t}$ and $c^0_{2,t}$ stand for the total molar concentrations of the individual compounds, the hydrolysate and the surfactant, respectively, needed to produce the same interfacial tension (reduction).

3. Results and discussion

3.1. *Enzymatic hydrolysis process*

The starch acetates of DS 0.02 and 0.1 were used. The DS value of 0.1 meets the JECFA standards of the maximum level of modifying groups content in food grade starches (FAO, 1997). Starch is an extremely high molecular weight material (of degree of polymerization DP of amylopectin fraction over 10^6 Da) (Swinkles, 1985). Acetylation of starch does not influence its molecular weight, so one can expect similar technical problems to take place during ultrafiltration of both materials (Le Thanh et al., 2007).

As shown in Fig. 1, the character of permeate flux vs. time curves depended on the transmembrane pressure (ΔP) applied. For the lowest ΔP value (0.05 MPa) the permeate flux was almost perfectly

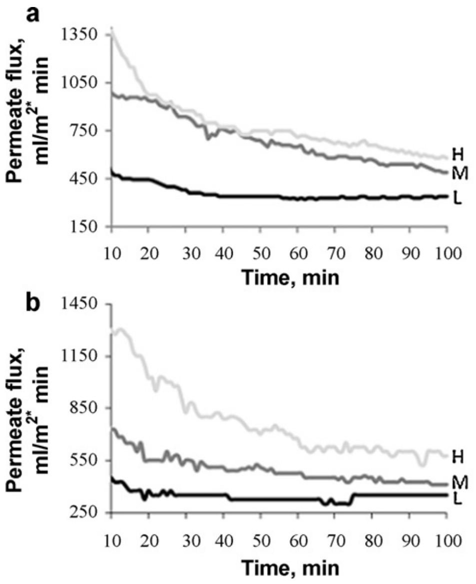


Fig. 1. Permeate flux vs. time of hydrolysis performed at different transmembrane pressures: L (–) 0.05 MPa, M (▬) 0.1 MPa and H (▬) 0.15 MPa for modified starches with DS (a) 0.02 and (b) 0.10.

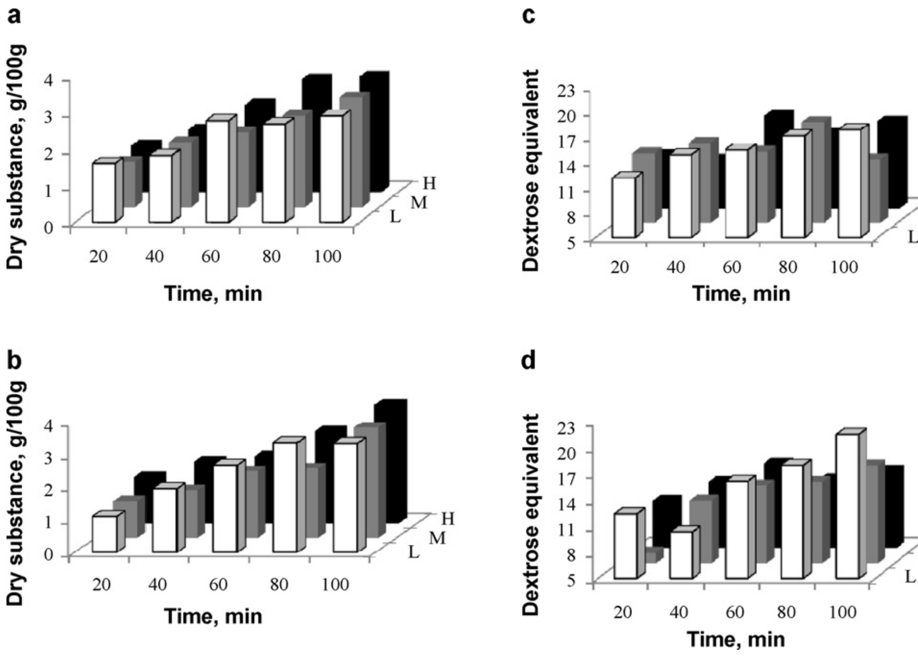


Fig. 2. Dry substance content (a and b) and dextrose equivalent (c and d) of hydrolysates contained in permeate fractions obtained during the hydrolysis of derivatives of acetylated starches with DS: (a) and (c) 0.02 and (b) and (d) 0.10.

stable over the whole measuring range. At higher ΔP, the character of the permeate flux vs. time curves changed. For the first 60 min of the hydrolysis process, as the reaction system was supplemented with a starch solution, a decrease in the relative permeate flux was observed.

For the next 40 min, when the reaction system was supplemented with water, the permeate flux stabilized. These observations suggested a steady state operation of the recycle membrane reactor system used in the later 40 min. However, as follows from Fig. 2, the productivity of CRMR, measured as the mass value dry substance, systematically increased, even when the reactor was supplemented with water.

The degree of substitution with acetyl groups did not influence the magnitude of the permeate flux (Fig. 1a and b), although a slight but noticeable decrease in the productivity of CRMR in hydrolysis of the derivatives of higher degree of acetylation (Fig. 2a and b) was observed. This phenomenon was independent of the transmembrane pressure applied. There are only a few reports on the susceptibility to enzymatic hydrolysis of acetylated starches. According to Gunaratne and Corke (2008), acetylation increases susceptibility of maize starch to α-amylase hydrolysis. On the other hand, *in vitro* digestibility of acetylated corn (Chung, Shin, & Lim, 2008) and potato starch (Le Thanh et al., 2007) has been found to be lower in comparison to that of the native counterparts. The influence of the presence of acetyl groups in the starch macromolecules on the susceptibility to amylolysis could be observed also as changes in dextrose equivalent (Fig. 2c and d). Dextrose equivalence is a measure of the number average molar mass or the degree of polymerisation (DP), according to the equation $\mathrm{DE} = 100/\mathrm{DP}$. In the membrane reactor used, a higher degree of substitution with acetyl groups resulted in a lower degree of saccharification. Dextrose equivalents of the products obtained were affected also by the operating parameters of the membrane reactor.

The higher the transmembrane pressure was applied, the higher value of dry substance in the permeate flux was determined. This phenomenon was a result of more efficient filtration at higher ΔP. Thus, with increasing transmembrane pressure, the efficiency of hydrolysis (defined as the amount of the product obtained) increased.

3.2. Surface/interfacial properties of hydrolysates of acetylated potato starch derivatives

3.2.1. Equilibrium surface/interfacial tension

For all products of enzymatic hydrolysis of acetylated potato starch derivatives obtained, the ability for reducing the surface and interfacial tension in the air/water and the oil/water systems was examined.

The experimentally obtained surface tension isotherms, for selected derivatives, are given in Fig. 3a and b. The figures presented allow a comparison of the surface (Fig. 3a) and interfacial activity (Fig. 3b) of hydrolysates obtained at different conditions applied during the progress of the hydrolysis process. Moreover the presented isotherms allow a comparison of the adsorption properties of hydrolysates and acetylated starch prior to hydrolysis. The results show that all hydrolysis products considered showed surface activity and reduced the surface tension in the air/water system as well as the interfacial tension in the toluene/water one. Almost all investigated hydrolysates were more surface active than the parent acetylated starch, i.e. the equilibrium surface tension of the air/water system and the equilibrium interfacial tension of the oil/water system were lower in their presence when compared to the systems with unhydrolysed derivatives. The products of enzymatic hydrolysis of the acetylated starches reduced the surface tension by about 20 mN/m.

The relation between the ability to lower surface tension and the degree of substitution was also significant. As follows from Fig. 3a, the products of hydrolysis of the acetylated derivative of higher degree of substitution (0.10) obtained at lower transmembrane pressures (*L*), reduced surface tension more effectively than that of the lower degree of acetylation (0.02). However, in the case of the products obtained at high ΔP, the influence of the degree of substitution with acetyl groups on the surface activity of derivatives in the toluene/water system was less significant.

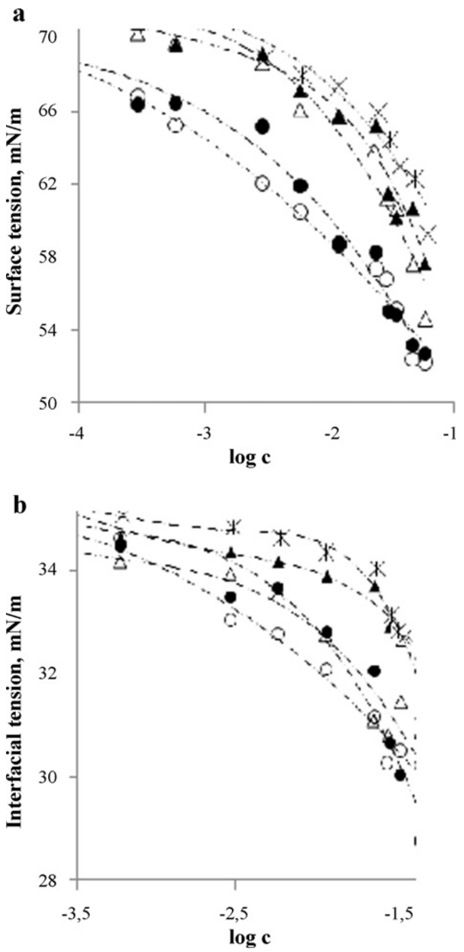


Fig. 3. Surface tension isotherms in the air/water system (a) and interfacial tension isotherms in the toluene/water system (b) for acetylated starch with DS = 0.02 (*) and hydrolysates: $L_{0.02}$ (▲), $L_{0.10}$ (△), $H_{0.02}$ (●) and $H_{0.10}$ (○).

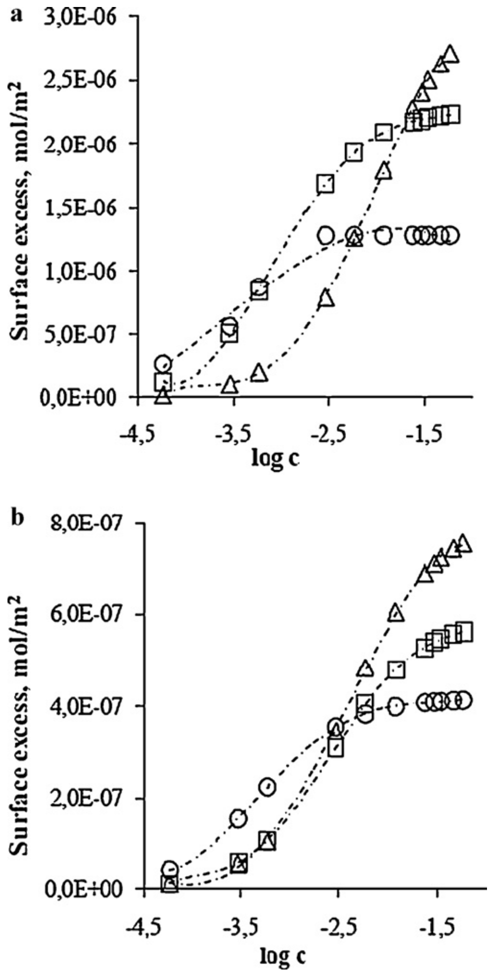


Fig. 4. Surface excess in the air/water system (a) and toluene/water system (b) for hydrolysates with DS = 0.1 obtained at: *L* (△), *M* (□) and *H* (○) transmembrane pressures.

It is worth noting that one of the most important parameters which affects the quality of the final products of enzymatic hydrolysis performed in the membrane reactor is the transmembrane pressure. Fig. 4 illustrates significant influence of the transmembrane pressure applied during the separation of final products of hydrolysis on their adsorption properties.

The effect of the magnitude of ΔP applied during the hydrolysis on the surface activity of the hydrolysates is clear. The products obtained in different conditions, i.e. at low and high transmembrane pressure, reveal quite different properties. The lower the ΔP, the weaker the ability to reduce both the surface tension at the air/water interface and the interfacial tension at the toluene/water interface (Fig. 3). Considering the two extreme transmembrane pressures of the UF process: *L* and *H*, the difference in the effect of reducing surface tension (around 5 mN/m) at the air/water phase boundary is clearly noticeable. The effect observed can be explained by the fact that as a result of increasing ΔP applied during the separation process, the fraction of molecules with shorter chains and increased tendency to reduce the surface tension grows. In other words, this observation pointed out that higher transmembrane pressure favored permeation of molecules with higher surface activity.

The surface/interfacial tension data obtained experimentally can be well fitted by the Szyszkowski equation. The adsorption parameters for exemplary systems are collected in Table 1.

The adsorption parameters allow characterisation of the surface properties of aqueous solutions of the hydrolysates of acetylated potato starch derivatives.

The data tabulated in Table 1 indicate that, in both systems considered (oil/water and air/water) the derivative $L_{0.1}$ formed the most densely populated interface adsorption layer. For $H_{0.1}$, the values of the surface excess in the saturated interface Γ^{∞} were near three times (the air/water interface) or about twice (oil/water interface) lower than those obtained for $L_{0.1}$ derivatives. This is consistent with the values of ΔG_{ads} and indicates that the derivatives with increased fractions of molecules with short chains obtained using higher transmembrane pressures during the separation process yielded higher adsorption tendency. The same relations can be observed for the surface activity in the oil/water system as well as in the air/water case (Fig. 3 and Table 1).

The surface excess isotherms shown in Fig. 4 indicate that, in both systems considered the saturation at the interface took place at a relatively low concentration of the hydrolysates used. They also indicate that the higher ΔP used for the separation of the hydrolysates from the reaction mixture, the lower the value of the surface excess (surface concentration) at the saturated oil/water or air/water interface.

For the derivatives filtered at ΔP equal to 0.05 MPa, their bulk phase concentration required for the saturation of the interface is higher for the DS 0.1 hydrolysates. Adsorption efficiency of the components of the filtrate flux was high; however the mobility of the

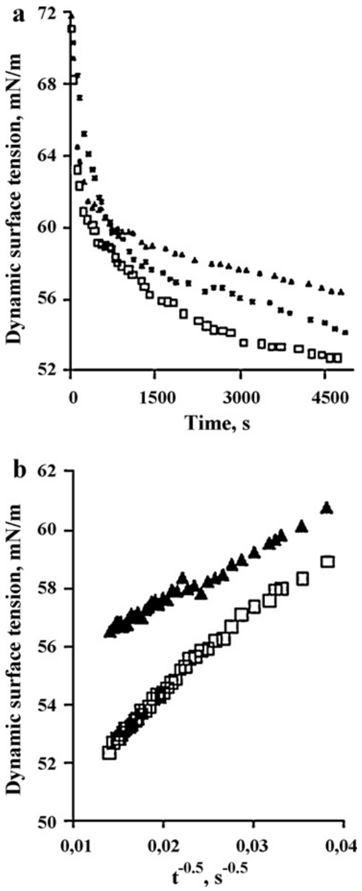


Fig. 5. Dynamic surface tension vs. time (a) and reciprocal square roof of time (b) for $M_{0.02}$ at $c = 0.1\%$ (w/w) (■), $M_{0.02}$ at $c = 0.01\%$ (w/w) (▲), $M_{0.10}$ and $c = 0.01\%$ (w/w) (□) in the air/water system.

particles from the bulk phase to the interfacial region was relatively low. A possible explanation is that the low transmembrane pressure yielded less short-chain molecules and thus a decrease in the adsorption effectiveness was observed. As a result the filtrate components formed a very densely populated layer at the interface, but their tendency for adsorption was rather low. The concentration of the surface layer decreased with increasing degree of starch substitution.

Accurate analysis of the adsorption parameters estimated for the toluene/water system was difficult to perform because of the fact that the reduction of the interfacial tension was only a few mN/m. However, the results indicated that an increase in the acetyl group content was consistent with the increase in adsorption effectiveness as well as with the higher tendency to adsorb at the liquid/liquid interface.

3.2.2. *Dynamic surface tension*

The influence of the bulk concentration of the solution and the degree of substitution of the hydrolysates on the character of dynamic of adsorption at the air/water interface was studied.

The results of measurements of dynamic surface tension of the acetylated starches are given in Fig. 5a. The starch samples lowered the surface tension very quickly, within approximately 20 s, and then after approximately 5 min the observed changes of surface tension were insignificant.

The experimentally obtained dynamic surface tension data allowed estimation of the diffusion coefficient D of the hydrolysates of the acetylated starch.

The values of diffusion coefficients were calculated according to Eq. (4).

In accordance with Eq. (4) the values of the dynamic interfacial tension were plotted as a function of the reciprocal square root of time ($t^{-1/2}$). The straight lines obtained shown in Fig. 5b, representing all investigated systems, indicate that the analyzed adsorption process is diffusion controlled, and the diffusion coefficients can be calculated according to Eq. (4).

However, it is worth mentioning that the quantity of D calculated according to Eq. (4) is equivalent to the conventional diffusion coefficient if there is no energy barrier between the surface and the subsurface (Tornberg, 1978). In general, the value of D is a quantity characteristic of both diffusion and the crossing of the barrier. Moreover, in Eq. (5) it is assumed that the diffusion is ideal, i.e. the solute–solute interactions are negligible. In very dilute systems this approximation would be justified.

The influence of concentration on the value of diffusion coefficient D is insignificant. On the other hand, the value of diffusion coefficient is affected by the degree of acetylation of the hydrolysates, however the order of magnitude of D for the derivatives of different degrees of acetylation is the same. The value of D estimated for $M_{0.02}$ derivative ($0.91 \times 10^{-7}\ \mathrm{cm^2/s}$ and $0.99 \times 10^{-7}\ \mathrm{cm^2/s}$ for 0.01% (w/w) and 0.1% (w/w) concentration, respectively) was more than three times as high as that of $M_{0.1}$ ($0.24 \times 10^{-7}\ \mathrm{cm^2/s}$ for 0.01% (w/w) concentration). In other words, the results obtained suggest that the hydrolysates of the DS 0.1 starch gave lower diffusivity values than the hydrolysates of the DS 0.02 starch.

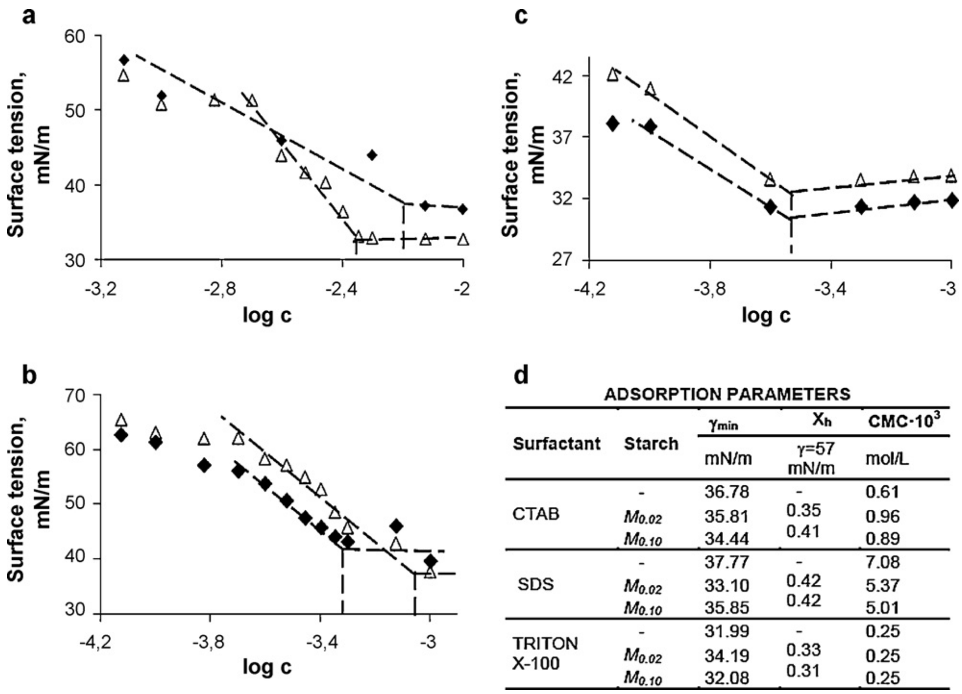
3.2.3. *Surface activity of binary systems*

It is well known that starch is able to form complexes with amphiphilic substances (Bravo Rodriguez, Jurado Alameda, Martinez Gallegos, Reyes Requena, & Gracia Lopez, 2008; Penfold, Thomas, & Taylor, 2006; Tian et al., 2009; Tomasić, Tomasić, Smit, & Filipović-Vinceković, 2005). From the application point of view, these complexes have a major influence on the starch retrogradation process.

To establish the influence of the surfactant added on the magnitude and type of interaction between hydrolysates of acetylated starch and an amphiphilic substance added to the solution, some additional investigation for multi-component systems was performed.

Three types of binary mixtures with three surfactants used as amphiphilic additives were investigated: an anionic surfactant SDS, a cationic surfactant CTAB and a nonionic one TRITON X-100. As components of these binary systems, two derivatives of the modified starch hydrolysates, $M_{0.02}$ and $M_{0.1}$, were considered. In all mixtures studied, the molar ratio of the surfactant to the starch components was 1:6 mol/mol. The surface tension isotherms for binary systems obtained, illustrated in Fig. 7, indicate that the addition of a hydrolysate to a water solution of surfactant, irrespectively of the type of surfactant used, caused a change in the surface activity of a given mixed system.

As known from the study of mixed protein–surfactant systems (Kotsmar et al., 2008; Pradines, Krägel, Fainerman, & Miller, 2009), the formation of complexes whose interfacial properties differ from those of the individual components and depend on the composition of the formed aggregates is possible. The structure of these complexes is strongly influenced by the concentration of surfactant. At low surfactant concentrations, only electrostatic interaction occurs, which leads to the formation of more hydrophobic complexes with stronger adsorption properties than those of the native protein (Alahverdjieva, Fainerman, Aksenenko, Leser, & Miller, 2008). When the concentration of surfactant increases, hydrophobic interactions occur between the protein and the surfactants, which consequently leads to less surface active complexes.



ADSORPTION PARAMETERS

Surfactant	Starch	γ_{min} mN/m	X_h γ=57 mN/m	CMC·10^3 mol/L
CTAB	-	36.78	-	0.61
	$M_{0.02}$	35.81	0.35	0.96
	$M_{0.10}$	34.44	0.41	0.89
SDS	-	37.77	-	7.08
	$M_{0.02}$	33.10	0.42	5.37
	$M_{0.10}$	35.85	0.42	5.01
TRITON X-100	-	31.99	-	0.25
	$M_{0.02}$	34.19	0.33	0.25
	$M_{0.10}$	32.08	0.31	0.25

Fig. 6. Surface tension isotherms for (♦) surfactant solution and (△) mixed system $M_{0.02}$:surfactant for (a) SDS, (b) CTAB, (c) Triton X-100 and adsorption parameters of mixed starch–surfactant compositions at molar ratio 6:1 mol/mol (d).

Close to the critical micelle concentration (CMC) of surfactant, replacement of complexes by the surfactant is observed due to strong competitive adsorption (Mackie, Gunning, Wilde, & Morris, 2000).

Thus, in the mixed systems containing surfactant and hydrolysate considered, complexes can also be formed by electrostatic and/or hydrophobic interactions. These complexes can have totally different adsorption properties than the individual components.

As one can see the magnitude of the surface tension reduction was affected by the type of surfactant as well as by the degree of esterification of the hydrolysates used (Fig. 6). Depending on the character of the surfactant, different point shift of CMC was observed for the two-component mixtures. The values of CMC and other adsorption parameters for systems with surfactant used alone and for mixed systems are presented in Fig. 6d. The comparison of the estimated values of CMC for the surfactant solutions alone with their binary mixtures with starch hydrolysate revealed the influence of starch addition on the CMC point shift.

For the mixtures of CTAB + starch, the CMC point was shifted towards higher concentrations. For the pure surfactant solution its critical micelle concentration was 0.61 mmol/dm^3. For the two-component mixtures, the value of CMC reached 0.96 mmol/dm^3 for CTAB + $M_{0.02}$ and 0.87 mmol/dm^3 for $M_{0.10}$ + CTAB. The addition of a hydrolysate to a solution of the anionic surfactant SDS also resulted in a CMC point shift, however in the opposite direction, towards lower concentrations. Quite different behaviour was observed for mixtures of the non-ionic surfactant and starch as the addition of a hydrolysate to the Triton X-100 solution did not cause any shift of the CMC point.

For mixtures of starch and ionic surfactant, a significant effective reduction of surface tension was observed. For example, the γ_{CMC} value for SDS solution was 37.8 mN/m. After addition of $M_{0.02}$, the surface tension value γ_{CMC} lowered to 33.1 mN/m. When the added hydrolysate was $M_{0.10}$, i.e. the higher acetylated derivative, the γ_{CMC} value was lowered to 35.8 mN/m.

The molar fraction of the hydrolysate (X_h) at the saturated air/water interface in all mixed systems considered, calculated according to Eq. (5), was 0.35–0.42. Thus, as follows, for all binary systems studied the bulk composition was different from that the adsorption layer. In the bulk, the excess of starch was 6 mol/mol, whereas at the interface, the acetylated derivatives occupied no more than 40% of the surface area.

3.3. Micelle size distribution (MDS)

Taking into account the interactions between different types of emulsifiers and stabilizers on the stability of food emulsions, studies of the size of micelles formed by starch preparations investigated and their hydrolysates in aqueous solution were undertaken (Bruce, Berne, & Pecora, 2000; Dickinson, 2009; Huang, Kakuda, & Cui, 2001). The results of MSD measurements are presented in Fig. 7a and b.

The micelles size distribution of the starch preparations obtained was similar to that of commercial maltodextrin. It should be stressed that both commercial maltodextrin of DE 8.5 and the hydrolysates of acetylated starch preparations studied were obtained as products of enzymatic hydrolysis with alpha-amylase as enzymes.

The MSD curves illustrated in Fig. 7 revealed 2 intense bands assigned to small micelles (of average diameter 10 nm) and large micelles of average diameter around 200 nm. The observed shift of the bands relative to their positions in the spectrum of commercial maltodextrin is affected by the presence of functional groups incorporated into the macromolecules of starch during the esterification process. Similar behaviour of the hydrolysates was observed in our previous work, concerned with the products of hydrolysis of doubly modified starch (Prochaska et al., 2009).

However, it should be noted that the character of the MSD curves obtained for investigated hydrolysates is strongly affected by the degree of substitution (DS) of a given hydrolysate, i.e. the amount of acetyl group present in the macromolecule of hydrolysate. This

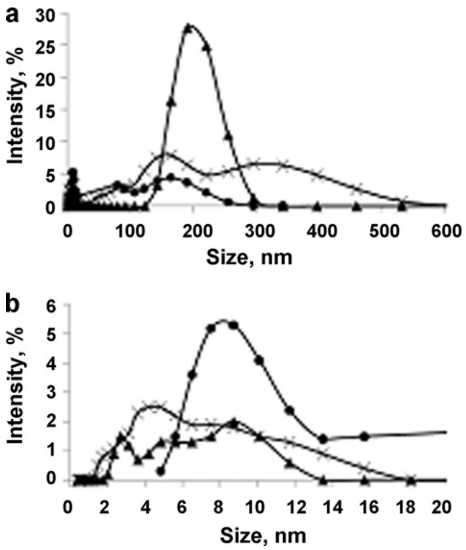


Fig. 7. Micelles size distribution for hydrolysates of $M_{0.02}$ (x) and $M_{0.10}$ (▲) and for commercial maltodextrin (●) in the range of (a) 0–600 nm and (b) 0–20 nm.

effect of DS is observed especially in the micelle size distribution of large aggregates.

Thus, a comparison of the surface activities of the hydrolysates studied and the measurements of MSD allowed drawing a conclusion that the degree of substitution of the hydrolysates of acetylated derivative is a parameter determining both their ability to reduce the surface tension at the air/water interface as well as the size of aggregates formed in the aqueous solutions.

4. Conclusions

It was found that the starch samples modified by acetylation were susceptible to enzymatic hydrolysis in the a continuous recycle membrane reactor (CRMR); however, a higher acetyl group content results in a decrease in the susceptibility to amylolysis. The fouling phenomena as well as the productivity of the CRMR, measured as the DE value and dry substance content in the permeate flux depended on the applied transmembrane pressure ΔP. Moreover, increasing ΔP caused a decrease in the dextrose equivalence of the products.

All hydrolysates of acetylated starch decreased the surface/interfacial tension both in the air/water and oil/water systems. The efficiency and effectiveness of adsorption of the hydrolysates were affected by the degree of substitution in the macromolecules of starch as well as by the value of the transmembrane pressure used for UF separation during the process of hydrolysis. As to mixed hydrolysate:surfactant systems, the addition of a hydrolysate to the aqueous solution of a surfactant caused an increase in the surface activity of the two-component systems probably as a result of complex formation which revealed totally different adsorption properties than those of the components. The same effect was observed in all binary systems investigated, independent of the character of the surfactant studied. Moreover, for all two-component systems studied the bulk composition was significantly different from that of the adsorption layer formed at the air/water interface.

However, the changes in the CMC point as well as the CMC point shift direction depended on the type of surfactant used. In the mixed systems with the nonionic surfactant, only an additional effect in the surface tension reduction was observed.

Micelle size distribution of the hydrolysates in aqueous solutions was similar to that of commercial maltodextrin. However, a strong effect of the degree of substitution of the starch derivative by acetyl groups on the course of MSD curves was observed.

The results obtained indicated that both the ability to reduce the surface tension at the air/water interface as well as the size of aggregates formed in the aqueous solutions are affected by the value of DS of a given hydrolysate, i.e. the total amount of acetyl groups present in the molecule of the product of hydrolysis of the acetylated derivative.

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Glossary

0.05 and 0.1: degree of acetylation of starch
$Ac_{0.02}$ *and* $Ac_{0.1}$: unhydrolysed acetylated starch
$L_{0.02}$, $L_{0.1}$, $M_{0.02}$, $M_{0.1}$, $H_{0.02}$, $H_{0.1}$: hydrolysates of acetylated starch
CTAB: cetyl trimethylammonium bromide (cationic surfactant)
SDS: sodium dodecyl sulfate (anionic surfactant),
Triton X-100: octyl phenol ethoxylate (nonionic surfactant)
A_{min}: surface area for statistical molecule adsorbed at saturated interface
c: bulk concentration (% w/w or mol/L)
$c^0_{2,t}$: total molar concentrations of surfactant,
$c^0_{1,t}$: total molar concentrations of hydrolysate,
CRMR: continuous recycle membrane reactor
D: diffusion coefficient (cm^2/s)
DE: dextrose equivalent
DLS: dynamic light scattering method
DS: degree of substitution
H: high transmembrane pressure (0.15 MPa)
L: low transmembrane pressure (0.05 MPa)
M: medium transmembrane pressure (0.1 MPa)
MSD: micelles size distribution
ΔP: transmembrane pressure (MPa)
R: gas constant (J/mol deg)
T: temperature (K)
X_h: surface molar fraction of hydrolysate
α: molar fraction of hydrolysate in the bulk solution
γ: surface/interfacial tension (mN/m)
γ_0: surface/interfacial tension for the distilled water (mN/m)
Γ: surface excess (surface concentration) (mol/m^2)
Γ^∞: surface excess at the saturated interface (mol/m^2)