Desalination and Water Treatment
www.deswater.com

14 (2010) 94–100
February

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doi: 10.5004/dwt.2010.1014

The influence of types of dual modified starches on the enzymatic hydrolysis in the continuous recycle membrane reactor

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Received 12 July 2009; accepted 4 November 2009

ABSTRACT

The aim of work was to study the influence of the degree of substitution of acetylated oxidized starch (E-1451) on the process of enzymatic hydrolysis in a continuous recycle membrane reactor (CRMR).

Three kinds of E-1451 starch preparations with different degrees of carboxyl group substitution (0.04, 0.11 and 0.5%) and with different degrees of acetylation: 0.5% and 2.5% (3%) were examined. The waxy corn modified starch was also investigated.

Hydrolyses were performed in the CRMR equipped with an ultrafiltration module containing a ceramic membrane of tubular configuration. The membranes used were characterised by the following parameters: a length of 0.3 m, external diameter of 10 mm, three channels and a molecular weight cut-off of 50, 15 or 8 kDa. During the process the UF module was working in three variants: with a membrane of 15 kDa or a set of two membranes: 50/15 kDa and 15/8 kDa. The hydrolysis process was performed over a period of 150 min at a temperature of 60°C, at the transmembrane pressure of 0.5 MPa and with a 0.3 ml of enzyme preparation BAN 480 L per 1 kg of dry starch. It was stated that the efficiency of the separation process as well as the effectiveness of hydrolysis process was affected by the type of modification and the degree of substitution of starch.

Keywords: Membrane reactor; Membrane fouling; Membrane permeability; Enzymatic hydrolysis

1. Introduction

Starch is a common and widely-used raw material for food production and industrial application and is used both natural and modified form [1–5]. Native starches present limitations that reduce their use at the industrial level [3]. To improve their quality, native starches need to be modified: physically (using heat or moisture), chemically or enzymatically. Chemical modification inserts various substituents to starch macromolecules, which significantly change the functionality of the starch preparations (e.g. by etherification, esterification, or cross-linking). Acid or enzymatic hydrolysis of starch brings decreasing (decomposition) of the molecular weight of starch chains during the process [4].

It is obvious that the enzymatic hydrolysis of native and modified starch not only contributes to decreasing the molecular weight of starch chains, but also changes physical and chemical properties—mostly viscosity, as well as the surface properties of hydrocolloid solutions.

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Presented at the Fourth Membrane Science and Technology Conference of Visegrad Countries (PERMEA 2009), Prague, 7-11 June, 2009.

Starches modified doubly by oxidation and acetylation are commonly used and applied in the food industry as stabilizers, thickeners, binding agents and emulsifiers. Additional hydrolysis of modified starches might significantly broaden the area of functional food additives and starch products application.

Classical enzymatic hydrolysis processes are implemented in periodical bioreactors in a two step procedure. This method has many disadvantages: large capacity of the reactor, substrates spend a long period in the bioreactor and the low quality of the obtained products [6]. The application of the membrane reactor allows continuous process realization, repeatedly reducing the time of hydrolysis, moreover it produces an enzyme-free and high quality product, where additional purification is not required [6–8]. Additionally, it allows recovery of the enzyme and enabling a reduction in the global cost of hydrolysis.

The capability of an efficient application of the continuous recycle membrane reactor (CRMR) for enzymatic hydrolysis of chemically modified starches, was the main object of the presented work. Moreover, the influence of the type of modification as well as the degree of substitution of acetyl groups in the macromolecules of starch on the adsorption activity for obtained product of hydrolysis in the air/water and oil/water systems were investigated.

2. Materials

Three kinds of commercial food grade oxidized starches: Pudding powder *P*, Gel-forming food starch *G* and Lubox *L*, with 0.04%, 0.1% and 0.5% of carboxyl groups content respectively, produced by WPPZ Luboń (Poland) were examined. All oxidized starches were additionally acetylated to get doubly modified starches of different degrees of acetylation: 0.5% and 2.5% for *P* and *G* as well as 0.5% and 3% for *L*. An acetylated oxidized waxy corn starch *C* was also investigated.

An enzyme preparation- BAN 480L (bacterial α-amylase produced by selected strains of *Bacillius amyloliquefaciens*), kindly provided by Novozymes company (Denmark), were used as the hydrolytic enzyme.

3. Methods

Modified E-1451 starch gruels were prepared by heating starch solutions to 90°C, cooking for 60 min, then adjusted to pH = 6.5 with citric acid. The initial concentration of starch preparation solutions was equal to 5% (*P* and *C*) or 10% (*L* and *G*) depending on their viscosity. During the process the reaction mixture was vigorously mixed (250 rpm). Next, 0.3 ml of enzyme BAN 480L per 1 kg of solid substrate was added. The hydrolysis was performed at a temperature of 60°C during 180 min. For first 30 min hydrolysis was carried out on the bypass without simultaneous separation of products. Main hydrolyses were performed in the continuous recycle membrane reactor (CRMR) consisting of a reaction vessel BioFlo III 5L (New Brunswick Scientific Co., Inc. USA), tubular and ceramic membrane (Tami Industries, France) and rotary lobe pump (Johnson, USA). The scheme of the reactor was presented in our previous paper [5].

The membrane separation (attendant the hydrolysis) was carried out in one of two UF units containing a ceramic membrane of molecular weight cut-off 50 kDa (membrane A) or 15 kDa (membrane B), with a surface area of 0.087 m^2 and length of 0.25 m. The membrane filtration was performed at a transmembrane pressure of 0.5 MPa. The separation process lasted for 2 h (initial 60 min using membrane A of 50 kDa and then 90 min using the membrane B).

The filtrate fractions were systematically collected while the retentate was recycled back. After completion of the process, the collected permeate fractions and retentate samples were examined. The amounts of reducing groups (Dextrose Equivalent) of the sugar mixtures were determined by modified *School-Rogenbogen* method (PN-78/A-74701). The dry matter content of permeate and retentate as well as moisture content of products obtained by spray drying were determined by an oven-drying method (PN-EN ISO 1666:2000; PN-78/A-74701) [5,9].

The viscosity measurements were performed with the VT 550 Haake Viscotester (Germany) at 60°C. The equilibrium surface/interfacial tension for the investigated doubly modified starches and their enzymatically hydrolysed products (i.e., the dried solutes of permeate) were measured by the *du Noüy ring method* with a KRÜSS K12 tensiometer (Germany). The measurement of the dynamic surface tension was carried out with an automated tensiometer TRACKER I.C. Concept Instrument, France [10,11]. All measurements were carried out at 21 ± 0.1°C. The standard deviation of the surface/interfacial tension measurements was about 0.05 mN/m. Distilled water with conductivity equal to 3 μS was used as the aqueous phase, and toluene (Aldrich) was used as the oil phase in the interfacial tension measurements. Although toluene cannot be used as a food additive, it was applied in our investigations as a model organic phase due to its well defined chemical structure and physicochemical properties.

The experimentally obtained surface tension data were fitted by the Szyszkowski equation [12]. The

Table 1
Viscosity of chemically modified starch gruels (500 rpm)

Starch preparation	Viscosity η [Pa*s]	
	Low acetylated	High acetylated
Pudding	0.184	0.201
Gel-forming	0.016	0.014
Lubox	0.003	0.003

adsorption coefficients of Szyszkowski isotherm were used to estimate the surface excess at the saturated interface (Γ^{∞}) and the Gibbs free energy of adsorption ($\triangle G_{\mathrm{ads}}$). These values were calculated in the same way as was described in our previous paper [13]. The adsorption parameters were estimated with assumption that an anhydroglucose unit is an equivalent of one mole of investigated starch or its hydrolysed products.

4. Results and discussion

4.1. Viscosity of solutions of starch preparations

The type of modification caused different rheological properties of investigated starch preparations. As can be seen in Table 1, the degree of oxidation, 0.04% for Pudding starch, 0.1% for Gel-forming starch and 0.5% for Lubox starch, played a mine part in creating the rheological behaviour of starch gruels. The higher the degree of oxidation the lower the viscosity of starch preparation. The acetyl group substitution had a secondary meaning.

Enzymatic hydrolysis of modyfied starches brought about dramatic changes of viscosity of starch solutions during the process (see Table 2).

The viscosity of the initial starch gruel (0 min) is much higher than the viscosity of the gruel after first 30 min of hydrolysis i.e. before UF sepatation (30 min) and than the viscosity of retentate fraction received in progress of filtration process.

Overly high viscosity of starch solutions caused significant membrane fouling (see Fig. 1).

To prevent the fouling, for first 30 min the hydrolysis was progressed without concurrent separation on UF module. The use of a membrane with larger cut-off at the beginning of the separation of products of hydrolysis was also recomended.

4.2. Influence of the degree of substitution on the hydrolysis process

The influence of the degree of substitution of starch derivatives on the effectiveness of the enzymatic hydrolysis expressed by the changes of mass flux of permeate during the process is presented in Fig. 2. The mass flux of permeate was calculated according to the Eq. (1).

$$\dot{m} = \frac{V \times m_{*}}{A \times t \times V_{*}} \left[\frac{\mathrm{g}}{\mathrm{m}^2 \times \mathrm{min}}\right] \tag{1}$$

where $\dot{m}$—mass flux, V—volume (cm^3), A—membrane area (m^2), t—time (min), m—mass (g)

*Calculated according to PN-EN ISO 1666:2000; PN-78/A-74701.

The obtained results show that the effectiveness of hydrolysis process was affected by the type of modification, as well as the degree of substitution in the macromolecules of starch. However, the degree of acetylation of the investigated, chemically modified hydrolysed derivatives was a parameter of secondary meaning.

4.3. Efficiency of hydrolysis (dextrose equivalent; DE)

The conventional measure of hydrolysis productivity is a change of dry substance (DS) in the filtrate fraction (Fig. 3), whereas the measure of efficiency of starch hydrolysis is the DE of obtained products. The results presented in Fig. 3a show that for higher oxidized starch (*L*) we can observe significant increase of dry mass of filtrates, while for lower oxidized starch (*P*) the changes of amount of DS were much smaller (see Fig. 3b).

Fig. 3b also illustrated the impact of the type of origin of starch on the DS value of filtrate fractions obtained during the hydrolysis of chemically modified starches. Although the degrees of acetylation and oxidation are the same for both waxy corn and potato starches, the value of DS in filtrate flux is approximately 3 times bigger for waxy corn starch. The most likely reason for this behaviour is different

Table 2
Changes of the viscosity during the hydrolysis process of P 0.5% Ac starch

Time of hydrolysis (min)	0	30	60	90	120	150	180	210
η [Pa*s] (500 rpm)	0.184	0.0065	0.0038	0.0036	0.0037	0.0040	0.0038	0.0038

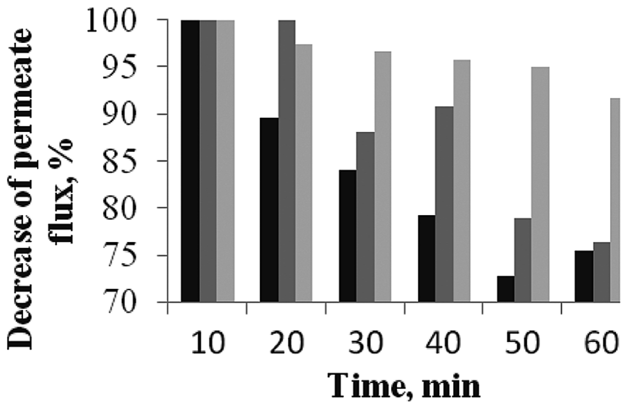

Fig. 1. Membrane fouling for: (■) Pudding; (■) Gel-forming and (■) Lubox 0.5% Ac starch (set of 15/8 kDa membranes).

amylopectin content in each of the considered starches (it is higher in waxy starches).

The influence of the carboxyl and acetyl groups' content was observed in the case of the changes of the DE values listed in Table 3.

Thus, the obtained results indicated that the planning of the hydrolysis process, realized in the CRMR, the type of starch (the origin) and the kind of modification need to be taken into account. Additionally, the membrane selection, and the viscosity of the initial starch gruels are also important parameters.

Considering the DE values, it can be concluded that the higher degree of acetylation of derivatives is usually caused by higher reducing group content in the permeate flux. In other words the efficiency of enzymatic hydrolysis is affected by the degree of acetylation of starch preparation. The same effect was observed independently of the set of membrane used in separation process.

4.4. Surface activity

Surface-active hydrocolloids may act as emulsifiers and emulsion stabilisers. In order to determine their potential as stabilisers or emulsifiers, surface and interfacial tension for water soluble E-1451 starch hydrolysates were measured [14–16]. Moreover, measurements of the surface/interfacial tension give brief information of the structure of the adsorption monolayer [17–19].

The obtained results (Fig. 4) show that all considered products of the hydrolysis process of the acetylated oxidised starches were surface active and reduced the surface tension in both the air/water and oil/water systems.

The investigated products of the hydrolysis of the acetylated Pudding starch decreased the surface tension from 72 to about 40 mN/m (air/water system) and interfacial tension from 45 to 24 mN/m (toluene/water system). The adsorption parameters are shown in Table 4. The density of a saturated interface, $_{\infty}$, is one of the parameters which can characterise the interfacial activity of the compound and can be

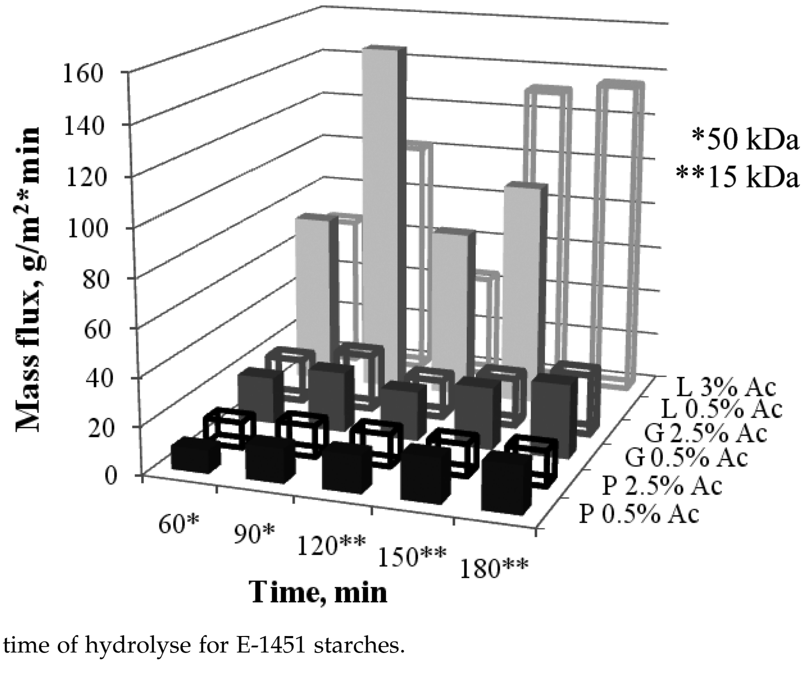

Fig. 2. Mass flux versus time of hydrolyse for E-1451 starches.

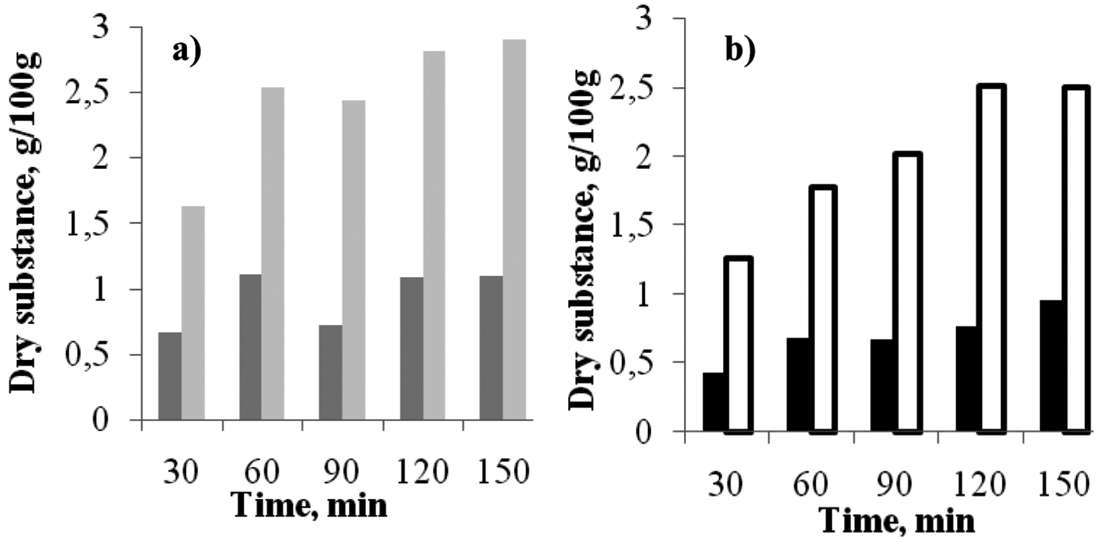

Fig. 3. The change of the content of the dry substance in the filtrate fractions during the enzymatic hydrolysis of: (■) Pudding starch; (□) Oxidised waxy corn starch; (▨) Gel-forming starch; (▒) Lubox starch; initial concentration of 0.5% Ac starch preparation solutions: (a) 10%, (b) 5%.

Table 3
DE values of hydrolysis products obtained after 150 min of the process

Starch preparation	Dextrose equivalent (DE)			
	Membrane 15/8 kDa		Membrane 50/15 kDa	
	"P"	"G"	"P"	"G"
Low acetylated	21.9	22.1	19.0	23.2
High acetylated	30.3	24.4	25.6	20.9

used as the measure of the adsorption effectiveness at the fluid/fluid interface. However, the values of the free energy of adsorption suggested the tendency to adsorption.

The surface activity in the air/water system was two times higher than in the oil/water system. In the other words, the surface layer at the air/water interface was much more populated in the case of the adsorption in the second system.

On the other hand, examination of the corresponding values of the free energy of absorption ΔG_{ads} show that in all considered systems the products of

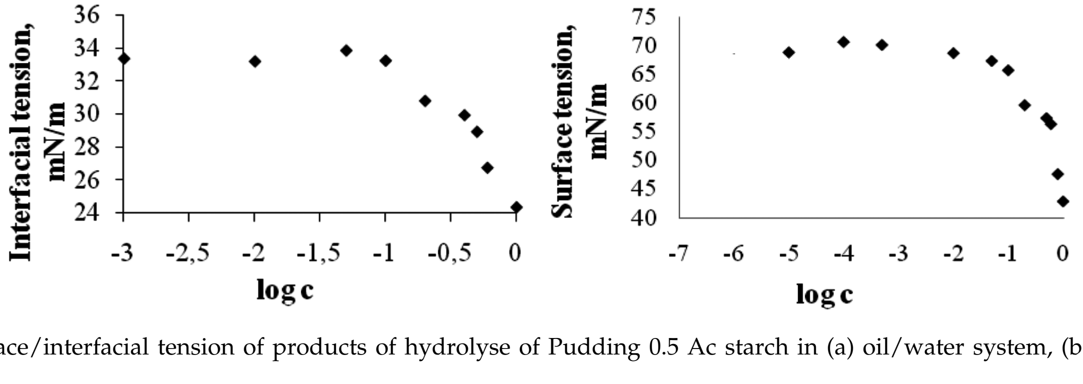

Fig. 4. Surface/interfacial tension of products of hydrolyse of Pudding 0.5 Ac starch in (a) oil/water system, (b) air/water system.

Table 4
Adsorption parameters of considered hydrocolloid

	Adsorption parameters	
Parameter	Air/water system	Toluene/ water system
$-\Delta G_{ads}$ (kJ/mol)	10.2	9.1
$\Gamma^{\infty}\cdot 10^{6}$ (mol/m^2)	6.9	3.5

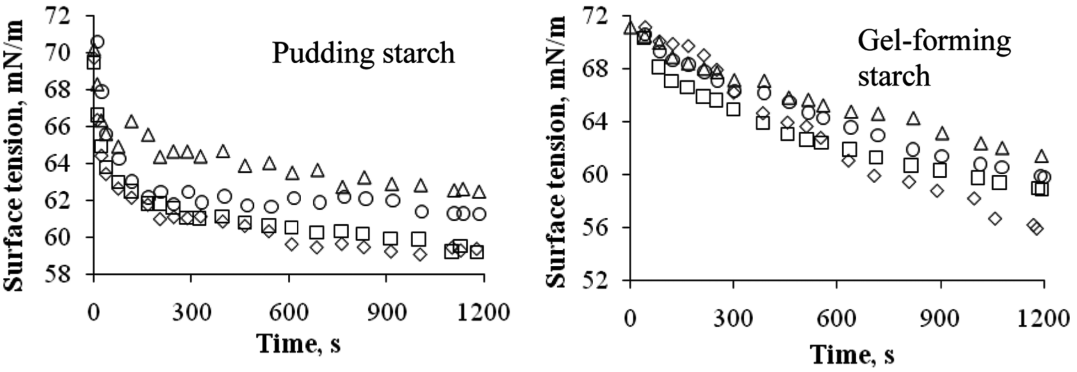

Fig. 5. Dynamic surface tension isotherms for permeate fraction after: (◇) 30 min, (□) 60 min, (o) 120 min and (Δ) 150 min of UF separation as a function of interfacial age.

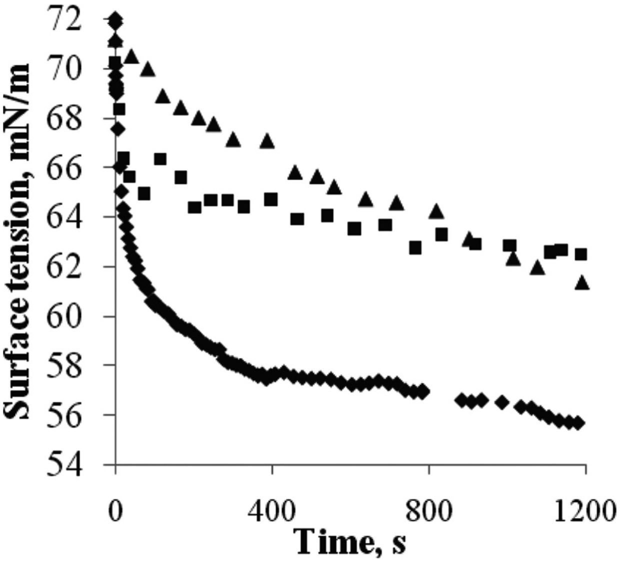

Fig. 6. Dynamic surface tension isotherms of permeate fraction of: (■) Pudding, (▲) Gel-forming, and (◆) Lubox E- 1451 0.5 Ac modified starches as a function of interfacial age.

hydrolysis of E-1451 starch are characterised by quite high tendency for adsorption.

The dynamic surface tension of the hydrocolloids is given in Fig. 5.

The surface properties of the obtained filtrates were changing during the hydrolysis and the separation processes. By controlling the time of hydrolysis it is possible to get many products of different surface properties.

The investigations of dynamics of adsorption at the air/water interface showed a significant influence of the degree of oxidation of starch on the run of dynamic curves (see Fig. 6).

For instance, the hydrolysed *P* starch lowered interfacial tension quicker and more rapidly, within approximately 200 s to a constant value of 60 mN/m, whereas in the case of hydrolyzed *G* starch a constant surface tension is reached after more than 1,200 s.

5. Conclusions

- Planning the hydrolysis process, realized in the CRMR, the type of starch (the origin) and the kind of modification need to be taken into account.
- The effectiveness of the membrane process is strongly influenced by the rheological properties determine fouling phenomena in the reaction system.
- The degree of substitution of acetyl groups in the macromolecules of starch does not influence on the effectiveness of hydrolysis.
- The application of the CRMR for the enzymatic hydrolysis of E-1451 starches allowed products to be obtained which reveal surface activity.

Acknowledgments

The authors thank Professor Lewandowicz and Dr Le Thanh for helpful discussion, constructive comments and suggestions. The work was supported by 32-067/09-DS and by KBN 1073/B/HO3/2009/37.

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