Preparation and properties of propylene oxide and octenylsuccinic anhydride modified potato starches

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Abstract Starch was dually chemically modified for developing food-grade ingredients of lower digestibility and their properties were compared to those of single modified and native starches. Hydroxypropylation with propylene oxide (HP) followed by esterification with octenylsuccinic anhydride (OSA) of potato starch (P-native) produced derivatives with lower digestibility than esterification solely with OSA. The dextrose equivalent, maltose and glucose contents, which were used as the main indicators for in vitro digestion, were lower for modified starches. P-HP_{0.2}-OSA_{0.0200} derivative was the least digestible; the glucose and maltose contents were lowered by 28.3 and 42.1% compared to P-native. The aggregation behavior of enzymatically hydrolyzed starch derivatives was studied in aqueous solution by employing the fluorescence probe and dynamic light scattering techniques. The critical aggregation concentration for OSA modified and dually modified starches varied from 1.2 to 3 g/L and from 0.125 to 0.48 g/L, respectively, depending on the degree of OSA substitution. The study showed that above a critical concentration the hydrolyzates of modified starches tend to form the aggregates with different properties

depending both on the degree of OSA substitution and chemical structure.

Keywords Potato starch · Dually modified · Critical aggregation concentration · Fluorescence probe technique

Introduction

Modified starches, when used as food ingredients, present several benefits. For instance, they are effective in preparation of oil-in-water emulsions, which are used for encapsulating sensitive food ingredients; whereas native granular starch has limited solubility in water, does not contain lipophilic groups and therefore does not form emulsions.

Specialty starches for emulsification are hydrolyzed n-octenylsuccinic anhydride (OSA) modified starches produced from waxy corn starch; their brand names are HiCap, Capsul, NLoc, EmCap, and Cleargum. OSA imparts hydrophobic octenyl groups providing to starch amphiphilic character. OSA-modified starches have been used as substitutes for various food grade emulsifiers, including gum Arabic, fats, proteins; however, they are of interest for any area where stable emulsions are required (Sweedman et al. 2013).

Hydroxypropylated starch (HP) is another important product group in food applications. Etherification of starch with propylene oxide in the presence of alkaline catalyst imparts improved shelf life, freeze/thaw stability, low tendency for retrogradation, cold water swelling, and reconstituting properties to a formulated product. The HP groups weaken or disrupt the internal bond structure that holds the starch granules together and influence physico-chemical properties of products depending on the starch source, reaction conditions, the type of substituent groups

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employed, and the extent of substitution (Kaur et al. 2004; Schmitz et al. 2006). The HP-starches have been prepared from different sources, including potato (Kim et al. 1992; Choi and Kerr 2004; Hung and Morita 2005); their physicochemical properties have been intensively studied as a function of the degree of molecular substitution. The effect of hydroxypropylation on rheological properties of starches was also examined (Chun and Yoo 2007; Lee and Yoo 2011). Moreover, HP corn and amaranth starch hydrolyzates were reported as wall materials in microencapsulation of flavours (Kshisagar and Singhal 2008).

Currently HP (E1440) and OSA-modified (E1450) starches are approved by EU as carriers in all food additives at *quantum satis* levels (EU 2011). However, the reports on the properties and applications of dually modified starches are rather scarce. The combination of crosslinking and hydroxypropylation (Singh et al. 2007; Zhao et al. 2012), hydroxypropylation-crosslinking and acetylation (Raina et al. 2006, 2007), acid hydrolysis and hydroxypropylation (Fouladi and Nafchi 2014) were reported. Han and BeMiller (2007) used a combination of hydroxypropylation and esterification with OSA in water, and heat treatment to produce the products of corn starch consisting of low rapidly digesting starch (RDS) and high resistant starch (RS) fractions. HP–OSA–starch had lower RDS content than OSA–starch; the digestion of heated HP–OSA–starch decreased, while the content of RS increased by 41%. Consequently, heated HP–OSA–starch is comparable to commercial RS products and has an advantage because of its functionality. Rosu et al. (2013) reported that OSA-modified HP potato starch synthesized in dimethyl sulphoxide was able to solubilize organic pollutant benzo[a]pyrene. However, no consistent studies on aqueous synthesis and the properties of propylene oxide and OSA-modified potato starches have been reported until now.

This study aimed at synthesizing dually chemically modified starches for food applications and evaluating their properties. The primary objective was development of HP/O SA-modified potato starches and comparison of their properties with OSA-modified and native potato starches. A secondary objective was to prepare enzymatically hydrolyzed modified starches and to study their aggregation behavior in water in order to determine the critical aggregation concentrations (CAC), and the properties of the aggregates formed by the interaction of the polysaccharide chains.

Materials and methods

Materials and chemicals

Native potato starch (P-native) was supplied by JSC “Stumbras” Antanavas starch plant (Kaunas, Lithuania).

Propylene oxide (99%), 2-octen-1-ylsuccinic anhydride (mixture of *cis* and *trans*, 97%), 1,2,3-indantrione monohydrate (95%), propylene glycol (99.5%), potassium sodium tartrate tetrahydrate ($\geq 99\%$), copper (II) sulfate pentahydrate ($\geq 99.0\%$), pyrene ($\geq 99\%$), α -amylase from *Aspergillus oryzae* (30 U/mg), pepsin from porcine gastric mucosa (600–1200 U/mg), pancreatin from porcine pancreas, bile extract porcine were purchased from Sigma-Aldrich. α -Amylase preparation Liquozyme X (200 KNU(T)/g) from Novozymes A/S (Denmark) was used for liquefaction of starch derivatives.

Starch modification

Hydroxypropylation (P–HP)

P-native, 400 g dry weight of starch (dws), was dispersed in 750 mL distilled water containing Na_2SO_4 (20 g/100 g dws) and the pH adjusted to 11.3 with 1 M NaOH. After adding 12% (dws) of propylene oxide the reaction vessel was sealed, placed in a 25 °C water bath for 24 h with stirring. The slurry was neutralized to pH 5.5 with 1 M HCl, the granules washed with water, methanol and acetone and dried at 40 °C. The HP content was determined spectrophotometrically (Johnson 1969) from a calibration curve prepared using aqueous propylene glycol solutions and P-native as a blank. The concentration of propylene glycol was converted to HP-group equivalents, and the degree of substitution, DS_{HP} , in moles of substituent per mole of anhydroglucose unit, was calculated (Kshisagar and Singhal 2008).

Octenylsuccinic anhydride modified starch (P–OSA)

P–OSA and P–HP–OSA starches of varying molar substitution were prepared by reacting OSA with P-native and P–HP, respectively. Starch (300 g dry weight (dw)) was dispersed in 366 mL distilled water, pH adjusted to 8 with 1 M NaOH and OSA (1, 2 and 3% dws) added continuously agitating at ~ 20 °C and pH 8.5. After 6 h slurry was neutralized to pH 5.5 with 1 M HCl, the granules washed and dried as for P–HP. OSA content was estimated by titration with aqueous 0.05 M I_2 solution using unmodified starch as a blank. The degree of substitution, DS_{OSA} , was defined in moles of substituent per mole of anhydroglucose unit.

Characterisation of modified starches

The FTIR spectroscopy of solid samples was performed by using Frontier FT-IR spectrometer (Perkin Elmer) equipped with ATR accessory. Pasting properties were evaluated by Micro Visco-Amylo-Graph[®] (Brabender). Starch (5 g

dw) and distilled water (95 mL) were added to the aluminum container and subjected to the controlled heating–cooling cycle under constant shear: 30 °C, 1 min; heating from 30 to 95 °C at 4 °C/min; 95 °C, 10 min; cooling to 50 °C at 6 °C; 50 °C, 10 min. Peak viscosity, temperature at which it was reached, hot paste viscosity, breakdown, setback and final cold paste viscosity were recorded.

For differential scanning calorimetry (DSC) in a Perkin Elmer DSC8500 analyzer the samples were prepared at 1:1 dws/water ratio, hermetically sealed, and after 12 h heated from 30 to 80 °C at 5 °C/min in nitrogen atmosphere. Onset, peak, completion temperatures, and enthalpy of gelatinization were calculated.

Digestibility studies

The in vitro digestibility of starches was assessed according to the method of Miller et al. (1981), which was later modified by Rufián-Henares and Delgado-Andrade (2009), and supplemented with an oral step (Pastoriza et al. 2011).

For oral digestion 14 mL solution (32.5 mg α -amylase in 25 mL 1 mM CaCl_2 , pH 7.0) were mixed with 20 g starch paste (1 g dw) and incubated at 37 °C for 30 min by shaking at 95 rpm. For gastric digestion the sample after oral step was acidified to pH 2 with 1 M HCl; 1.2 mL porcine pepsin solution (0.4 g pepsin in 2.5 mL 0.1 M HCl) added and incubated for 2 h at 37 °C by shaking at 95 rpm. For intestinal digestion the pH was adjusted to 6 with 1 M NaHCO_3 and 3 mL porcine pancreatin-bile solution (0.1 g pancreatin and 0.625 g bile extract in 25 mL of 0.1 M NaHCO_3) added. Afterwards the pH was raised to 7.5 with 1 M NaHCO_3 and the samples were incubated at 37 °C for 2 h to complete the intestinal digestion phase.

After all digestion steps the enzymes were inactivated by heating for 4 min in a water bath, and then the samples were cooled in an ice bath. Finally, they were centrifuged at 10,000g for 15 min to separate soluble fraction. The dextrose equivalent (DE) was determined (Schoorl 1996) and expressed as reducing sugars. Dry matter was determined with KERN MRS 102-3 moisture analyzer, dry soluble solids were measured by Atago PAL-3 refractometer. Soluble digestion fraction was filtered through 0.4 μm filters and analysed with UPLC-MS/MS to determine glucose and maltose.

UPLC-TQ-S-MS analysis

Separation was performed on an Acquity BEH, Amide, 2.1×100 mm, 1.7 μm column (Waters) using acetonitrile/ultra-pure water/ NH_4OH (75/25/0.05%) mobile phase. The compounds were detected by single ion recording at m/z 341 and 179 on a Waters TQ-S triple quadrupole mass detector in

negative ionization mode with capillary and cone voltages 2500 and 25 V, respectively. Cone and desolvation temperatures were 150 and 250 °C, respectively. Nitrogen was used as cone (120 L/h) and desolvation gas (900 L/h). The constituents were identified by comparing their retention times and spectra with references. The concentration, in mg/g dw, was determined from calibration curves drawn using 12.5–400 $\mu\text{g/mL}$ solutions of glucose ($y = 503.784x - 4501.97$; $R^2 = 0.991$) and maltose ($y = 8601.86x + 31177.1$; $R^2 = 0.989$).

Enzymatic hydrolysis of modified starches

Modified starches were suspended in distilled water at 30% (w/w), and pH adjusted to 6 with NaOH or HCl; then α -amylase preparation Liquozyme X (0.08 g/kg dws) was added and the resultant slurry gelatinized at 80 °C in a water bath by mixing. After that the solution was kept in an 80 °C oven for 1.5 h for complete gelatinization. Then the temperature was increased to 98 °C for 10 min to inactivate the enzyme. The samples of slurry were precipitated in acetone; the hydrolyzed modified starches were separated by centrifugation, washed three times with acetone and air-dried. The purified products were used to prepare the samples for fluorescence probe and particle size measurements. The DE values were used to follow the extent of hydrolysis.

Fluorescence probe experiments

Steady-state fluorescence spectra were recorded on a Perkin Elmer LS-55 luminescence spectrometer using pyrene fluorescence to monitor the properties of hydrolyzed samples. A known concentration of pyrene was dissolved in diethyl ether; an aliquot of this solution was transferred to a volumetric flask and allowed to evaporate to dryness. Then hydrolyzed samples of modified starches from concentrated solutions/dispersions were added to the aqueous solutions of probes under magnetic stirring, placed in an ultrasonic bath for 10 min to facilitate dispersion of the probe throughout the samples and the fluorescence spectra were recorded. The ratio between the fluorescence intensities of peaks 1 (372–373 nm) and 3 (383 nm) of the pyrene emission spectrum (10^{-5} M), (I_1/I_3), was used to evaluate the polarity of local environment and to determine the critical aggregation concentration (CAC). Pyrene was excited at 338 nm and the emission recorded from 360 to 600 nm.

Dynamic light scattering (DLS)

Particle size distribution in aqueous solutions of hydrolyzed modified starches was determined by DLS on a

DelsaTM Nano C particle size analyzer (Backman Coulter), which uses photon correlation spectroscopy measuring the rate of fluctuations in laser light intensity scattered by a particle. The non-negative least-squares algorithm was used to analyze DLS data. Particle size measurements were reported as volume distribution and defined as the average particle diameters, which were mean values of 10 replicate measurements made on at least three freshly prepared samples.

Statistical analysis

All analyses were replicated for at least three times and all data are reported as mean $\pm$ SD using MS Excel 2003. Data were statistically handled by one-way analysis of variance (ANOVA, vers. 2.2). Duncan's multiple-range test was applied for the calculation of the significant differences among the values of characteristic parameters at probability level $P < 0.05$.

Results and discussion

Synthesis and characterization of OSA-modified starches and dually modified starches

P-HP with DS_{HP} of 0.2 was obtained by the etherification of P-native with propylene oxide in the presence of alkaline catalyst. P-OSA and P-HP-OSA ($DS_{OSA} = 0.007$ – 0.02) (Table 1) were obtained from the esterification reaction between OSA and hydroxyl groups of starch or P-HP, respectively. FTIR spectroscopy confirmed the modification: the spectra of P-HP showed characteristic absorption bands of starch and the band around 2970 cm^{-1} corresponding to the stretching of the $\text{CH}(\text{CH}_3)$ group, while for P-OSA-modified samples some characteristic peaks were at 1721 and 1573 cm^{-1} indicating stretching ester carbonyl group and asymmetrical stretching vibration of carboxylate group, respectively (see Suppl. Figs 1, 2 in Online Resource 1).

Pasting and gelatinization properties were mostly related to the properties of starch granules, which were affected by the modification. The gelatinization temperatures were derived from DSC curves (see Suppl. Figs 3, 4 in Online Resource 2): for P-HP_{0.2} they decreased from 65.0 (P-native) to $62.4\text{ }^\circ\text{C}$, while viscosity peak increased from 576 to 659 BU (Table 1). Gelatinization temperature decrease was consistent with fewer crystals being present after modification and with cooperative melting process enhanced by added swelling (Kaur et al. 2004), while viscosity increase of potato starches after hydroxypropylation was also reported previously (Kaur et al. 2004; Kim et al. 1992). Incorporation of 1.56% of bulky OSA groups

Table 1 Composition and pasting characteristics of modified starches

Sample	HP content (%)	DS_{HP}	OSA content (%)	DS_{OSA}	Reaction yield (%)	Gelatinization temperature ($^\circ\text{C}$)	Peak viscosity (BU)	Hot paste viscosity (BU)	Breakdown viscosity (BU)	Final viscosity (BU)	Setback viscosity (BU)
P-native	—	—	—	—	—	65.0 ± 0.3^f	576	274	302	496	222
P-OSA _{0.0076}	—	—	0.97 ± 0.01^a	0.0076 ± 0.001^a	97.2 ± 1.0^d	59.9 ± 0.5^d	645	341	304	524	183
P-OSA _{0.0123}	—	—	1.56 ± 0.02^b	0.0123 ± 0.001^d	78.2 ± 1.0^e	59.1 ± 0.4^{cd}	720	387	333	526	139
P-OSA _{0.0200}	—	—	2.52 ± 0.04^d	0.0200 ± 0.001^c	84.2 ± 1.0^f	56.2 ± 1.0^b	623	276	347	403	127
P-HP _{0.2}	6.69 ± 0.01^a	0.2 ± 0.01^a	—	—	55.8 ± 0.1^a	62.4 ± 0.5^e	659	417	242	545	128
P-HP _{0.2} -OSA _{0.007}	6.69 ± 0.01^a	0.2 ± 0.01^a	0.91 ± 0.02^a	0.007 ± 0.001^a	91.0 ± 2.0^b	58.8 ± 0.2^c	675	332	343	497	147
P-HP _{0.2} -OSA _{0.015}	6.69 ± 0.01^a	0.2 ± 0.01^a	1.89 ± 0.05^c	0.015 ± 0.001^e	94.5 ± 2.5^d	56.9 ± 0.5^b	910	461	449	656	195
P-HP _{0.2} -OSA _{0.020}	6.69 ± 0.01^a	0.2 ± 0.01^a	2.66 ± 0.03^e	0.020 ± 0.001^c	88.7 ± 1.0^b	55.2 ± 0.3^a	763	227	536	329	102

Values within columns followed by the same letter (a–f) do not differ statistically at $P < 0.05$ (Duncan test)

decreased gelatinization temperature to 59.1 °C and increased peak viscosity to 720 BU, which was in agreement with the previously published results (Sweedman et al. 2013). Hydroxypropylation followed by octenylsuccinylation produced starches with lower gelatinization temperatures comparing to a single modification, indicating that incorporation of the HP and OSA groups creates disorder in granular structure, enabling granules to begin swelling at lower temperatures and to a greater degree. However, after increasing OSA content in P–OSA and P–HP–OSA to 2.5–2.7% both peak and final viscosity of the paste decreased, which was in agreement with the results of Wang et al. (2010) who also observed a noticeable drop of viscosity at DS greater than 0.016 for P–OSA potato starch.

In vitro digestibility of modified starch derivatives

Changes of DE values

The in vitro digestion method was applied for P-native, P–HP_{0.2}, P–OSA and P–HP–OSA starches using DE as the main indicator of digestibility (Table 2). The differences in DE values after 1st step were not remarkable, although in some cases significant. For instance, OSA-starches gave higher DE-values comparing with P-native, while hydroxypropylation (P–HP_{0.2}) had no effect. The addition of OSA groups into the P–HP structure resulted in a decrease of DE to 27.1 ± 0.5 (P–HP_{0.2}–OSA_{0.020}), which indicated about possible digestibility reduction. Quite similar tendencies were observed after the 2nd digestion step, whereas after the 3rd step the DE-values were lowered for all modified starches than P-native. The lowest DE was found for P–HP_{0.2}–OSA_{0.020} (lower by 15% comparing with P-native) indicating that this starch derivative might be the most resistant to the gastric enzymes.

Octenylsuccinylation reduced the enzyme-catalyzed degradation of starch and glycemic response (Wolf et al. 2001; Han and BeMiller 2007). It was explained that OSA-starch increased the production of breath hydrogen, which

resulted from modified starch not digested by the intestinal amylases undergoing at least partial anaerobic fermentation by colonic hydrogen-producing bacteria (Cummings et al. 1996; Heacock et al. 2004). If part of an OSA-starch preparation was RS, the available energy from carbohydrate in the product was reduced. Han and BeMiller (2007) reported that the OSA-starches had high percentage of RS (potato 33%; corn 22–29%) and high content of slowly digestible starch (SDS) (corn 25–36%); they suggested that OSA-starches can be a good source of SDS. It was reported that P-native contained 78% RDS and 18% SDS; octenylsuccinylation decreased RDS and increased SDS and RS content. Also, it was proved that HP/OSA modification of waxy corn starch was very efficient—RDS decreased 2.4 times, whereas SDS increased 1.8 times compared to native starch; RS comprised the highest percentage (40.7%) in the modified product. A low glycemic index starch was previously developed by partial α -amylase treatment of maize starch (Han et al. 2006).

In general, it could be concluded that modification with propylene oxide reduced the digestibility, while P-native was the most digestible. However, the addition of OSA groups into P-native did not reduce DE, while inclusion of OSA groups in P–HP_{0.2}–starch resulted in a significant decrease in indicating increase in resistance of derivative to enzymatic hydrolysis. P–HP_{0.2}–starch without OSA groups was less digestible ($P < 0.05$) than P-native.

Although the differences in the DE obtained in our study for the tested starch derivatives were not remarkable, the tendency of lower digestibility of modified starch derivatives may be clearly observed; e.g. OSA-modification and particularly dual HP/OSA-modification reduced the digestibility of P-native. It should be noted that selected enzymes and in vitro digestion method applied in our study was originally developed for assessing the release of antioxidants (Pastoriza et al. 2011); however, in general it simulated digestion conditions and may be applied for the in vitro simulation of digestion of other nutrients such as starch derivatives.

Table 2 The DE values of modified starches pastes after the different digestibility steps

Modified starch paste	1st step (oral)	2nd step (oral + gastric)	3rd step (oral + gastric + intestinal)
P-native	30.3 ± 0.7^b	33.5 ± 0.3^{def}	39.1 ± 0.5^g
P–OSA _{0.0076}	33.3 ± 0.7^d	34.2 ± 1.0^f	38.5 ± 0.5^f
P–OSA _{0.0123}	33.2 ± 0.3^d	33.8 ± 0.4^{def}	38.0 ± 0.4^e
P–OSA _{0.0200}	31.4 ± 0.3^f	32.6 ± 0.2^{bc}	36.7 ± 0.3^d
P–HP _{0.2}	30.0 ± 0.4^b	33.2 ± 0.1^{cd}	36.2 ± 0.2^c
P–HP _{0.2} –OSA _{0.007}	30.2 ± 0.4^b	32.0 ± 0.2^b	35.2 ± 0.2^b
P–HP _{0.2} –OSA _{0.015}	28.1 ± 0.5^e	29.7 ± 0.5^g	34.7 ± 0.4^b
P–HP _{0.2} –OSA _{0.020}	27.1 ± 0.5^a	27.9 ± 0.4^a	33.2 ± 0.2^a

Values within columns followed by the same letter (a–g) do not differ statistically at $P < 0.05$ (Duncan test)

Fig. 1 Effects of starch modification on the formation of maltose and glucose contents in absolute amounts mg/g dw (a) and relative to P-native = 100 (b) after digestibility. Values within columns followed by the same letter (a–f) do not differ statistically at $P < 0.05$ (Duncan test)

Changes in maltose and glucose contents during in vitro digestion

Further the effect of modification of maltose and glucose content was evaluated after in vitro digestion (Fig. 1a, b).

The concentrations of maltose and glucose after the 1st step were 271.1 ± 5.4 – 427.7 ± 7.0 and 27.3 ± 0.1 – 31.6 ± 0.2 mg/g dw, respectively. They were significantly lower for HP and P-OSA samples than for P-native. The lowest content of maltose was determined from P-OSA_{0.0200}–starch,

Fig. 2 I_1/I_3 ratio of the vibronic band intensities of pyrene (10^{-5} M) as a function of hydrolyzed P-OSA (a) and P-HP-OSA (b) concentration in water: P-OSA_{0.0076} and P-HP_{0.2}-OSA_{0.007} (white diamond), P-OSA_{0.0123} and P-HP_{0.2}-OSA_{0.015} (black circle), P-OSA_{0.020} and P-HP_{0.2}-OSA_{0.020} (white up-pointing triangle), respectively. Estimated CACs indicated with the arrows and presented as a function of degree of OSA substitution for P-OSA (white square) and P-HP-OSA (black up-pointing triangle) (c)

Table 3 CAC of hydrolyzed P-OSA and P-HP-OSA samples in water determined by pyrene fluorescence and I_1/I_3 ratio values after aggregation of macromolecules

Sample	CAC, (g/L) ^a	I_1/I_3 ^b
P-OSA _{0.0076}	3.0 ± 0.2^e	1.41
P-OSA _{0.0123}	1.8 ± 0.2^d	1.40
P-OSA _{0.0200}	1.2 ± 0.2^c	1.37
P-HP _{0.2} -OSA _{0.007}	0.48 ± 0.02^b	1.25
P-HP _{0.2} -OSA _{0.015}	0.19 ± 0.01^a	1.21
P-HP _{0.2} -OSA _{0.020}	0.13 ± 0.01^a	1.15

^a Values within columns followed by the same letter (a–e) do not differ statistically at $P < 0.05$ (Duncan test)

^b I_1/I_3 ratio after aggregation of macromolecules: P-OSA sample concentration was 10 g/l; P-HP-OSA – 1 g/l

while that of glucose from P-HP_{0.2}-OSA_{0.015}; comparing to P-native was lower by 36.6 and 13.6%, respectively.

The concentration of maltose after the 2nd step was 213.3 ± 2.3 – 292.9 ± 11.4 mg/g dw, i.e. up to 1.7-fold lower than after the 1st step; in all modified starches which was statistically different from P-native. The lowest amounts of maltose were obtained for P-HP_{0.2} (213.3 ± 2.3 mg/g dw) and HP-OSA derivatives with the highest OSA levels, P-HP_{0.2}-OSA_{0.015} (225.3 ± 8.6 mg/g dw) and P-HP_{0.2}-OSA_{0.0200} (234.7 ± 9.8 mg/g dw). The glucose content after the 2nd step increased more than twice, to 59.8 ± 2.4 – 66.2 ± 2.5 mg/g dw; however, the differences between the analyzed starches were statistically different from P-native. The lowest glucose concentrations were observed for P-HP_{0.2}-OSA_{0.0200} (59.8 ± 2.4 mg/g dw) and P-OSA_{0.0200} (63.3 ± 0.4 mg/g dw) samples.

The amounts of maltose and glucose after the final 3rd step of digestion were 174.4 ± 2.7 – 301.4 ± 9.3 and 54.3 ± 1.0 – 75.8 ± 1.1 mg/g dw, respectively (Fig. 1a). In general, the consistent dependence between maltose content changes during digestion and the type of modification was not observed; however gradual reduction in maltose content was observed for P-HP_{0.2}-OSA_{0.0200}: 309.3 ± 3.5 (1st) → 234.7 ± 9.8 (2nd) → 174.48 ± 2.7 (3rd) mg/g dw.

The highest content of glucose after the 3rd step was from P-native, indicating that it was most digestible. HP-modification reduced glucose content by 11.5%, which was not statistically different for P-OSA_{0.0076} (Fig. 1a, b). In general, dual HP/OSA-modification with the highest food grade OSA level was the most effective in terms of resistance to digestion: P-HP_{0.2}-OSA_{0.0200}-starch derivative was the least DS; the glucose and maltose content were lower by 28.3 and 42.1% compared to P-native (Fig. 1b).

Aggregation behaviour of enzymatically hydrolyzed modified starches in water

Enzymatic hydrolysis of modified starch derivatives

Enzymatic liquefaction and saccharification of starch increase DE of modified starches. The obtained hydrolyzates were isolated from the solutions for DE determination. The DE-values of hydrolyzed P-native, P-OSA_{0.0076}, P-OSA_{0.0123} and P-OSA_{0.0200} samples were 15.11 ± 0.51 , 13.18 ± 0.26 , 12.14 ± 0.57 and 10.28 ± 0.14 , respectively; whereas hydrolyzed P-HP_{0.2}, P-HP_{0.2}-OSA_{0.007}, P-HP_{0.2}-OSA_{0.015} and P-HP_{0.2}-OSA_{0.0200} had DE of 7.43 ± 0.28 , 5.97 ± 0.15 , 4.51 ± 0.23 and 5.48 ± 0.51 , respectively. In general, increasing OSA content resulted in the decrease of DE. Moreover, HP-modified starches demonstrated 2–2.75-fold lower DE-values than others. The obtained hydrolyzed hydrophobically modified starch derivatives could potentially serve as encapsulating agents for various low polarity substances, e.g. essential oils and other food ingredients; therefore the studies were continued for evaluating their aggregation behaviour in water and their suitability to solubilize the hydrophobes.

Fluorescence probe and aggregation behaviour studies

Information about association behaviour of enzymatically hydrolyzed modified starches in water was obtained from fluorescence measurements. The ratio of intensities (I_1/I_3) of the pyrene fluorescence emission spectrum reports the micropolarity and hydrophobicity of the region in which it was solubilized. Particularly it tended to concentrate inside the hydrophobic domains. The variation in I_1/I_3 indicated if pyrene molecules were within polar or apolar environment, i.e. the hydrophobic nature of these interactions (in case of the presence of apolar aggregates) (Evertsson and Nilsson 1999).

The I_1/I_3 was measured as a function of hydrolyzed modified starches concentration in water (Fig. 2). P-native was a hydrophilic system and I_1/I_3 of the emission spectrum remained about 1.9 in a wide concentration range. For the hydrolyzed P-OSA and P-HP-OSA I_1/I_3 depended on the degree of OSA substitution and chemical structure. Both samples present the same behavior, i.e. initially I_1/I_3 slightly decreases when the concentration increases and an inflection point can be identified at certain polymer concentration that is DS_{OSA}-dependent. CAC values were determined from the curves of I_1/I_3 versus polymer concentration (the arrows in Fig. 2a, b) as the intercept of the tangents to the curve before and after the point of inflection (Fisher et al. 1998). Data in Table 3 show that CAC decreases when DS_{OSA} of both single and dually modified starches

increases. In general, hydroxypropylation decreased the CAC by 10 times. It should be noted that DE after enzymatic hydrolysis of P-HP-OSA were 2–2.5 times lower, i.e. the chain length of macromolecules remained longer. Thus, the higher molecular weight led to the formation of hydrophobic microenvironments that occurred at lower polymer concentrations. Intra and/or intermolecular interactions were reported to be responsible for the formation of aggregates of natural polysaccharides such as pullulan (Akioishi et al. 1993). The solution behavior observed here by OSA and dually modified starches might be explained by postulating the existence of intermolecular interactions, since the content of hydrophobic OSA groups was not sufficient to promote intramolecular interactions. Meanwhile, for hydrolyzed P-HP_{0.2} sample the point of inflection

Fig. 3 Mean particle diameter as a function of hydrolyzed P-OSA (a) and P-HP-OSA (b) concentration in water: P-OSA_{0.0076} and P-HP_{0.2}-OSA_{0.007} (black diamond), P-OSA_{0.0123} and P-HP_{0.2}-OSA_{0.015} (black circle), P-OSA_{0.020} and P-HP_{0.2}-OSA_{0.020} (white up-pointing triangle), respectively

in the I_1/I_3 versus concentration curve (not shown) was not observed and CAC was not estimated, though the aggregation of macromolecules in water was noted, and the I_1/I_3 value equal to 1.55 was determined. Linear relationship can be obtained for the logarithm of CAC as a function of DS_{OSA} (Fig. 2c), which is usually found for the critical micelle concentration of nonionic surfactants. Therefore it was believed that the macromolecules tend to undergo intermolecular hydrophobic association and the observed behavior was similar to common surfactants.

At higher polymer concentrations (above CAC), when the presence of aggregates can be postulated, the polarity indicated by I_1/I_3 of pyrene showed to be dependent on both DS_{OSA} and chemical structure (Table 3). The I_1/I_3 for hydrolyzed P–OSA changed from 1.9 before aggregation to 1.37–1.41 after aggregation. Similarly I_1/I_3 for hydrolyzed P–HP–OSA reduced from 1.81–1.87 to 1.16–1.25. For the more OSA substituted samples, I_1/I_3 decreased to lower values than for the less substituted ones, indicating that higher DS_{OSA} values more hydrophobic aggregates were formed. For P–HP–OSA lower I_1/I_3 values were obtained indicating that for this system water was more effectively excluded from the inner region of the aggregates. Comparing I_1/I_3 for single and dually modified starches (Table 3) it may be observed that I_1/I_3 for P–HP–OSA were by 10–16% lower than for P–OSA.

The aggregation behavior of hydrolyzed P–OSA and P–HP–OSA in aqueous solutions was confirmed by particle size measurements (Fig. 3). It was found that by reaching CAC, P–OSA and P–HP–OSA molecules aggregate by forming nanoparticles that subsequently form large aggregates and precipitates. It could be noted that higher OSA content containing P–OSA and P–HP–OSA initially form slightly smaller particles. These results confirmed the formation of the particles of different nature after reaching CAC.

Finally, it may be concluded that investigated hydrolyzates of modified starch derivatives associated with water and form aggregates suitable for solubilization of hydrophobes.

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