Carbohydrate Polymers 84 (2011) 509–516

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

HPLC-ELSD analysis of six starch species heat-dispersed in [BMIM]Cl ionic liquid

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ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form
30 November 2010
Accepted 4 December 2010
Available online 14 December 2010

Keywords:
Ionic liquid
Starch
Microwave heating
Depolymerization
Dissolution
HPLC-ELSD

ABSTRACT

Acid hydrolysis of starch is a commonly used modification that reduces the molecular size and produces soluble starch. The thermal treatment of starch in 1-butyl-3-methylimidazolium chloride, [BMIM]Cl, ionic liquid (IL) provides an alternative method for that. Six native starches (wheat, barley, potato, rice, corn and waxy corn) were heat-dispersed under controlled microwave conditions or in oil bath into [BMIM]Cl. Starches and their degradation products were studied by using a HPLC-ELSD. ELS detector is a universal and response independent detector which after optimization produced a good response to small and large carbohydrates. However, it has rarely been applied to starch analysis. During heating in [BMIM]Cl components of native starches, amylopectin and amylose, started to degrade. The increasing reaction temperature and time intensified the degradation. The use of microwave heating speeded up dissolution and degradation of starch compared to the conventional heating. A separate acid catalyst was not required.

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1. Introduction

Starch is a low cost, natural, non-toxic, renewable and biodegradable carbohydrate, and therefore a desired starting material, e.g. for food and paper industry. It consists of two glucans: a practically linear amylose and a branched amylopectin (Pérez & Bertoft, 2010). Amylose contains primarily α-(1-4)-linked glucose units with total molar mass $\sim 10^5$–10^6 g mol^{-1}. The highly branched amylopectin, with molar mass $\sim 10^7$–10^9 g mol^{-1}, contains amylose like backbones and about 5% of α-D-(1-6)-linked branches. The sizes of these polymers depend on plant species and are only a rough estimation. The properties of starch can be chemically and/or physically modified depending on a target application. One modification is acid hydrolysis that reduces its molecular size and produces soluble starch for industrial applications (Daniel & Whistler, 1994).

Unlike other carbohydrates starch exists in granules, which vary in shape, size, composition and crystallinity depending on their biological source. This results in a very low solubility into any conventional solvent. The polymeric nature, intertwined structure and extreme difference in the size of its components complicate the analysis of starch. In addition, dispersed starch molecules tend to aggregate and easily precipitate on standing (Chen, Fringant, & Rinaudo, 1997).

A commonly utilized method in starch analysis is high-performance size-exclusion chromatography (HPSEC) combined with different detectors, such as multi angle laser light (MALLS) and/or refractive index (RI) (Chen & Bergman, 2007; Grant, Ostenson, & Rayas-Duarte, 2002; Suortti, Gorenstein, & Roger, 1998; You & Lim, 2000) or fluorescent detector (Charoenkul, Uttapap, Pathipanawat, & Takeda, 2006). Accurate separation of amylopectin and amylose is not clearly evidenced with HPLC, since it has not provided a clear resolution for those polymers (Batey & Curtin, 1996; Gérard, Barron, Colonna, & Planchot, 2001). Therefore starch is usually debranced prior to HPSEC/HPLC analysis and reported molecular masses of amylose and amylopectin based on calculations determined from the chain length distribution (Jiang, Campbell, Blanco, & Jane, 2010; Yoo & Jane, 2002). The lack of standards complicates the determination of the enormous molecular mass of amylopectin and amylose.

Comparison of analytical methods such as HPSEC, differential scanning calorimetry (DSC), and iodine binding procedure revealed that they all had some limitations in the determination of the content of amylose in starch (Zhu, Jackson, Wehling, & Geera, 2008). An IUPAC-sponsored team of specialists in the field has summarized problems related to current methods that there is no standardised reproducible method for quantifying molecular size of starch (Gidley et al., 2010). Therefore, the study of the analysis of starch is actual and important.

Prior to the analysis the native starch has to be completely dissolved (the granular structure is destroyed) without the degradation of the macromolecular structure of amylose and amylopectin. Most often starch granules are solubilized by using boiling water, 1 M NaOH, or aqueous dimethylsulfoxide (DMSO) (solutions 85–90%) (Mukerjea, Slocum, & Robyt, 2007).

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doi:10.1016/j.carbpol.2010.12.011

A use of microwave heating (MW) for dissolution of starch has been reported as well (Fishman, Rodriguez, & Chau, 1996; Kim, Huber, & Higley, 2006; Palav & Seetharaman, 2007; Roger, Bello-Pérez, & Colonna, 1999). Starch dispersions were usually heated by using a domestic microwave oven. Depending on the heating method (microwave vs. conductive heating) a different behaviour of starch was observed. In microwave treatment the vibrational motion and rapid increase of temperature resulted in granular rupture so that the polar polymers spilled into the medium rather than leached. The polar water molecules present in the crystalline regions of starch granules were also affected by microwaves. Their vibrational motion enhanced the rupture of granules as well (Palav & Seetharaman, 2007). Decreased molecular weight was also observed due to the molecular degradation.

1-Methyl-3-butylimidazolium chloride or dicyanamide (Biswas, Shogren, Stevenson, Willett, & Bhowmik, 2006) as well as 1-allyl-3-methylimidazolium formate (Fukaya, Sugimoto, & Ohno, 2006) ionic liquids (ILs) have been reported to dissolve starch. Ionic liquids are solvents that solely consist of ions. They have a negligible vapor pressure, high thermal stability and many of them are recyclable. Regardless of their high price ionic liquids have found their beneficial properties compared to common organic solvents. And hence are considered as green solvents (Wasserscheid & Welton, 2003). Recently they have been used to dissolve various carbohydrates (El Seoud, Koschella, Fidale, Dorn, & Heinze, 2007; Zakrzewska, Bogel-Lukasik, & Bogel-Lukasik, 2010). Until now dissolution as well as modification of cellulose in ionic liquid media (Li, Wang, & Zhao, 2008; Swatloski, Spear, Holbrey, & Rogers, 2002) has been more extensively studied than with starch. Dissolution of starch in [BMIM]Cl has been reported (Stevenson, Biswas, Jane, & Inglett, 2007) and depolymerization of starch in ionic liquid media without an acid catalyst has been patented (Myllymäki & Aksela, 2005) claiming a selective amylose depolymerization to monomeric products while amylopectin remained intact.

Here we report an analytical study of six native starches (wheat, barley, potato, rice, corn and waxy corn) carried out by a high-performance liquid chromatography equipped with an evaporative light scattering detector (HPLC-ELSD). The ELS detector is a universal and response independent detector that provides a good response for compounds that are less volatile than a mobile phase, but it has not been commonly used for starch analysis. Starches were heat-dispersed in ionic liquid media under controlled microwave conditions or in oil bath and starch components, amylose and amylopectin, as well as polysaccharides, generated during the heating, were studied. The effect of the temperature, heating time and method, microwave vs. conventional heating on starch in IL, was studied as well. Controlled microwave heating was performed with a microwave reactor from Biotage designed for synthetic purposes. To our knowledge, this is the first study where the synthetic microwave reactor is used for the dissolution of starch. For comparison, the same native starch species were DMSO-treated and analyzed.

2. Materials and methods

2.1. Materials

Wheat, barley and potato starches were obtained from Ciba Specialty Chemicals Oy (Raisio, Finland). Waxy maize was from TCI Europe nv (Zwijndrecht, Belgium). Corn or rice starches were purchased from Sigma Aldrich (Schnelldorf, Germany). All starches were oven dried before use (at 105 °C for 20 h). Ethanol (99.5%) was from Altia (Rajamäki, Finland). Distilled-deionized water was purified with a Simplicity 185 water purifier (Millipore). Dimethylsulfoxide (reagent grade) was obtained from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany) and used without further treatment. All other chemicals were purchased from Sigma Aldrich (Schnelldorf, Germany) and they were either trademarks from Fluka, Supelco or Sigma as mentioned in the following text. Polymer standards (BioChemika Dextran 5, 25, 50, 150 and 1400 kDa), D-(+)-glucose, amylopectin and amylose from potato starch (Fluka), maltopentaose (Supelco), butyl chloride and 1-methyl imidazole (Sigma). [BMIM]Cl was prepared according the literature (Huddleston, Willauer, Swatloski, Visser, & Rogers, 1998). [BMIM]Cl was dried before use overnight in high vacuum and stored in a desiccator. Water content of the IL determined by the Mettler Toledo DL36 Karl Fisher Coulometer was less than 0.1% (w/w). Anolyte and catolyte, Hydranal-Coulomat AG and CG, respectively were also obtained from Sigma.

2.2. Preparation of aqueous dispersions of starch for HPLC

Starch dispersions were prepared according the method reported by Yoo and Jane (Yoo & Jane, 2002). Starch (120 mg) was wetted with 1.2 ml of water and then dispersed in 10.8 ml of DMSO. The suspension was stirred while heating in a boiling water bath for 1 hour and then stirred for 24 h at room temperature. An aliquot (0.25 ml) of starch dispersion (1%, w/v) was mixed with five volume of ethanol (1.25 ml) to precipitate starch. The precipitate was centrifuged at the speed of 13 400 g for 10 min. The starch pellet was redissolved in boiling water (7 ml) and stirred for 30 min. The hot solution was filtered through nylon membrane filter (1.2 μm) and 100 μl of the starch solution (0.4 mg/ml) was injected into a HPLC equipment.

2.3. Dissolution of starch, subsequent depolymerization in ionic liquid and a sample preparation for HPLC

All dissolution experiments and depolymerization reactions were carried out under nitrogen atmosphere. Oven dried starch (100–150 mg) was added into hot [BMIM]Cl (2–3 g) with stirring and heated up to 100 °C to yield 5% (w/w) starch dispersion in IL. Depolymerization was carried out at temperatures 100, 125 and 150 °C. The reaction time ranged from 1 to 4 h. The reaction was monitored at different time intervals, 0.1 ml samples with concentration of 50 mg/ml were taken and precipitated by adding 0.8 ml of 99.5% ethanol and centrifuged at 13 400 g for 10 min. Pellet was washed with 1 ml ethanol and centrifuged again at 13 400 g for 10 min. Pellet was allowed to dry in a fume hood for 20 h. The dried sample was dissolved into 1.5 ml of distilled-deionized water and filtered through a nylon membrane filter (1.2 μm) and 100 μl of the solution (concentration of 0.3 mg/ml) was injected into the HPLC equipment.

2.4. Starch morphology

Dissolution of starch and disruption of starch granules in ILs were monitored with Zeiss ULTRA plus or Jeol JSM-6300F field emission scanning electron microscope (FESEM). Starch samples taken after the dissolution reactions were spread on a carbon tape attached on the aluminum disks. Samples were coated with 30 nm of platinum and observed at 500× and 1000× magnification.

2.5. Microwave reactor

All microwave-assisted reactions were performed using a single-mode microwave reactor, Emrys™ Synthesizer, Biotage, designed for a synthetic use. The reactor was equipped with a magnetic stirrer and pressure, temperature (IR detection) and power control.

2.6. Analytical methods

2.6.1. Composition of starch species determined with HPLC-ELSD equipment

The composition of starch species was determined with the HPLC instrument (Shimadzu) that consisted of three isocratic pumps (LC-10AD) equipped with a control unit (SCL-10A), a degasser (DGU-14A), an automatic sampler (SIL-10AD) and an evaporative light scattering detector (Polymer Laboratories PL-ELS 2100). A separation of amylopectin, amylose and hydrolyzed products was done by using a guard column, PolySep-GFC-P (35 mm × 7.80 mm, Phenomenex, Macclesfield, Cheshire, UK) and an analytical column, Poly Sep-GFC-P2000 (300 × 7.8 mm, Phenomenex, Macclesfield, Cheshire, UK). The temperature of the column oven (Shimadzu, CTO-10AS) was maintained at 40 °C.

Standard solution used for the method development contained amylopectin and amylose from potato starch, maltopentaose and glucose (1 mg of each solute in 1 ml of water). The ELS detector was optimized for the analytes and following parameters were used: 80 °C for an evaporative temperature, 50 °C for a nebulizer and 0.90 ml/min for a nitrogen gas flow. The mobile phase was distilled-deionized water passed through in-line membrane filters (0.45 μm Millipore, Bedford, MA) at a flow rate of 0.4 ml/min. The system was controlled and data was handled by using LC Solution program (LabSolutions 1.03 SP5 Shimadzu).

2.7. Calibration and method validation

The calibration curves were created for maltopentaose and amylose. Sample concentrations were 1, 2, 3 and 5 mg/ml and each concentration level was injected (10 μl) in triplicates. Amylose samples had to be done into 0.6 M NaOH solution otherwise amylose did not dissolve completely. Another calibration curve was determined by using more dilute samples with concentrations: 0.04, 0.12, 0.16 and 0.2 mg/ml for amylose and 0.1, 0.2, 0.3, 0.4, 0.7 and 1.5 mg/ml for maltopentaose so that use of NaOH was not necessary. Each concentration level was injected (100 μl) in triplicates. Samples were immediately analyzed to avoid retrogradation of amylose. The same standard concentrations were used for the determination of the method precision (interday). The estimation of the interday precision was carried out by analyzing standard samples once in a week or month depending on the need for the analysis.

3. Results and discussion

3.1. HPLC-ELSD method development

ELS-detector parameters were optimized with the standard solution that contained amylopectin and amylose from potato starch and sugars: D-(+)-glucose and maltopentaose 1 mg of each in 1 ml of water. Most appropriate parameters were 80 °C for an evaporative temperature, 50 °C for a nebulizer and 0.90 ml/min for a nitrogen gas flow. The lower (0.6 ml/min) or higher (1.2 ml/min) nitrogen gas flow rate decreased the sensitivity of the detector. Same was observed when the temperature of the nebulizer was decreased or increased (30 °C or 60 °C). The evaporative temperature was maintained as high as possible in order to evaporate mobile phase (water) properly before the sample detection.

Water was used as the eluent. Different flow rates (0.4, 0.5, 0.6 and 0.7 ml/min) were tested. The lowest flow resulted in best separation of amylopectin, amylose and sugars during the HPLC analysis.

The effect of the oven temperature was studied as well. It was first lowered from 60 to 40 °C and further to 35 °C but the separation of the components did not improve. Temperatures higher than 60 °C were not tested. Finally the temperature of the column oven was maintained at 40 °C in order to prevent gelatinization. The sample solution was kept dilute (<4 mg/ml) to prevent the aggregation of starch and blocking of the column. The pressure within the HPLC system did not rise after running over hundred samples and the performance of column was maintained. The % recovery of the HPLC-eluted 1400 kDa dextran standard and depolymerizated starch sample was about 80%. The recovery was consistent with the earlier report (83%, Chen & Bergman, 2007).

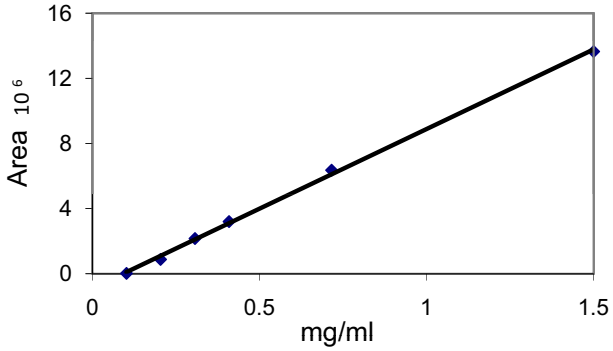

Fig. 1. Calibration curve of maltopentaose.

3.2. Calibration, response and molecular weight determination

The response determination of the ELS detector was carried out for maltopentaose and amylose. Both gave a good linearity of a response with the correlation coefficient of 0.998 and 0.972, respectively, for the range 1–5 mg/ml with 10 μl injections. Detection limit was 2 mg/ml for maltopentaose and 1 mg/ml for amylose. When more dilute samples (0.04–1.5 mg/ml) and larger injection volumes (100 μl) were used maltopentaose and amylose gave a linear response with correlation coefficients of 0.999 and 0.998 (Fig. 1). Detection limits for both compounds were 0.1 mg/ml. Calculations were based on the peak areas of the studied samples. Results from the detection of maltopentaose were very accurate and the accuracy of amylose was ±5%. The use of NaOH with more concentrated amylose samples was necessary to keep amylose dissolved completely. In dilute samples NaOH started to degrade amylose and therefore its use was excluded.

Experiments showed that it was more advantageous to detect analytes by using a dilute sample with a large injection volume than a more concentrated sample with a small injection volume. The quantitative analysis could be carried out with both maltopentaose and amylose.

The finding of large enough standards for the determination of molecular mass of starch is challenging. Dextrans were chosen to the purpose since they are water soluble and available in a wide range of molecular masses. Used standards gave guidelines to estimate the size of the depolymerized starch species. Polymer standards were analyzed with HPLC-ELSD chromatography. The retention time for amylopectin, amylose, 1400, 150, 50, 25 and 5 kDa standards, maltopentaose and glucose was 10, 16, 15.25, 15.5, 15.75, 16, 18, 22.5 and 25 min, respectively. HPLC-ELSD chromatograms of amylopectin, amylose, 150 and 5 kDa standards, maltopentaose and D-(+)-glucose are shown in Fig. 2. Accordingly to HPLC analysis the size of amylose was approximately in the range of 25–1400 kDa. The size of amylopectin was significantly higher than 1.4 MDa.

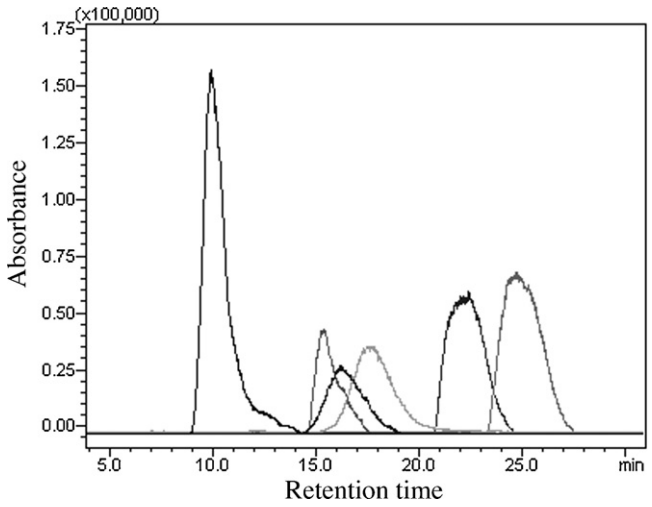

Fig. 2. HPLC-chromatograms of standards used for the determination of molecular weight. Peaks from left to right are (1) amylopectin, (2) 150 kDa dextran standard (3) amylose, (4) 5 kDa dextran standard, (5) maltopentose, (6) glucose.

3.3. HPLC study of native starches

The native starches were solubilized with a widely used DMSO treatment (Jackson, 1991) and analyzed with HPLC-ELSD. Chromatogram of the native barley starch is shown in Fig. 3. Amylopectin and amylose separated well. Their relative amounts were calculated from the peak areas of the HPLC-ELSD chromatogram (Table 1). The amounts are similar to the values reported in the literature (Zhu et al., 2008). The chromatograms of the corn, barley, rice and wheat starch each contained the peaks of amylopectin and amylose. The amylose peak area of the potato starch was smaller than expected. Possibly this was a consequence from the higher viscosity of the potato starch dispersion compared to the other starches. It was assumed that the separation of the main components (amylopectin and amylose of the potato starch) was not satisfactory and they eluted together. Waxy corn starch contained only the amylopectin fraction.

3.4. Dissolution of native starch species into ionic liquid

Dissolution of native starches (waxy corn, corn, rice, wheat, barley and potato) into ionic liquid was utilized by adding oven dried starch (100–150 mg) into a melted and stirred [BMIM]Cl (2–3 g) yielding 5% starch dispersion. The drier the IL was, the easier starch species dissolved into it. Halide anions have a strong tendency for hydrogen bonding with hydroxyl groups of polysaccharides. Hydrogen bonding disrupts existing inter- and intramolecular hydrogen bonds between hydroxyl groups of polysaccharide molecules and therefore favors their solubility into ionic liquids (Anderson, Ding, Welton, & Armstrong, 2002; Remsing, Swatloski, Rogers, & Moyna, 2006; Swatloski et al., 2002).

Table 1
Relative amounts of amylopectin and amylose in DMSO-treated starches according to the relative area HPLC-ELSD elution profiles and their retention times.

Starch	Amylopectin (%)	R_t (min)	Amylose (%)	R_t (min)
Waxy corn	100	10		
Corn	78	10	22	15.75
Barley	75	10	25	15.75
Rice	73	10	27	15.75
Wheat	67	12	33	16
Potato[a]	98	10	2	15.75

[a] The dispersion of the potato starch was cloudy.

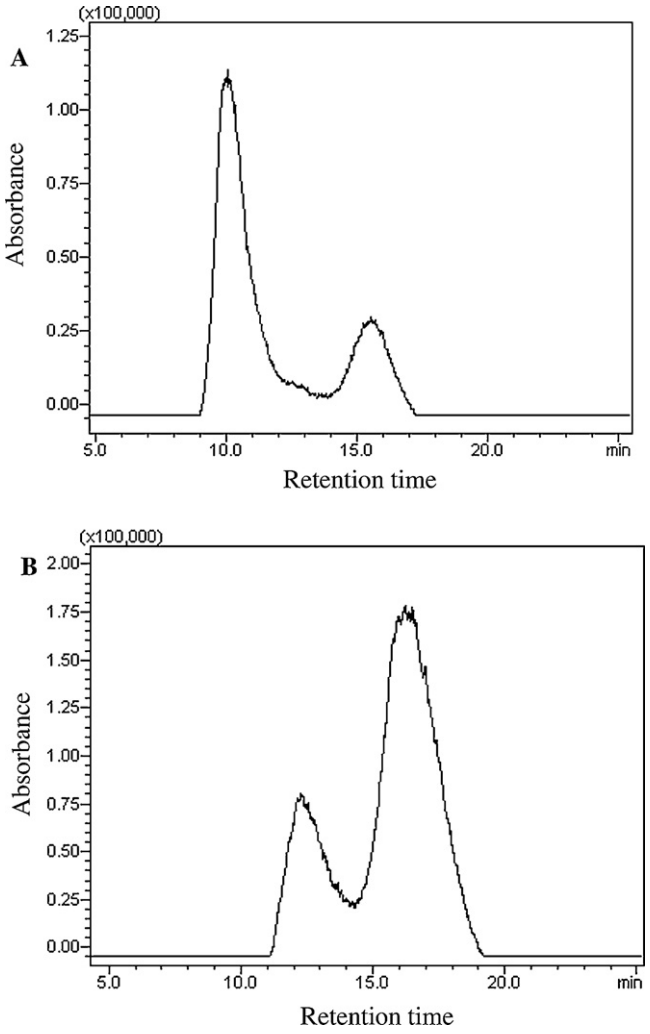

Fig. 3. The HPLC-chromatogram of native barley starch after DMSO treatment (A) and gelatinized barley starch after the dissolution in [BMIM]Cl (B).

While dispersions were heated in oil bath they formed a translucent gel, which after a while at 100 °C formed a transparent solution. The time for the gelatinization (1–13 min) and formation of a non-viscous, transparent solution (5–30 min) was characteristic for each starch species. Rice and corn starches with small-sized granules gelatinized faster than others (information shown as a Supplementary material).

Gelatinized starches were analyzed by HPLC-ELSD and their morphology was studied by using a scanning electron microscopy. Prior to analyses starches were precipitated with ethanol and dried. HPLC chromatograms showed that both amylopectin and amylose started to degrade during gelatinization. Chromatograms of DMSO-treated native barley and ionic liquid gelatinized barley starches are compared in Fig. 3. The scanning electron micrographs (SEM) of the native starches are shown in Fig. 4A–F. The SEM images of the gelatinized starches (Fig. 4G–L) showed that corn, rice, wheat or barley starch were not in a granular form, but waxy corn and potato starches contained some granules and were not fully gelatinized.

3.5. Comparison of microwave and conventional heating

Dissolution experiments were carried out by using a microwave heating. The temperature was set at 80 °C, which was the lowest temperature to achieve a proper mixing (the melting point of [BMIM]Cl is ca. 70 °C). All studied starch species dissolved into

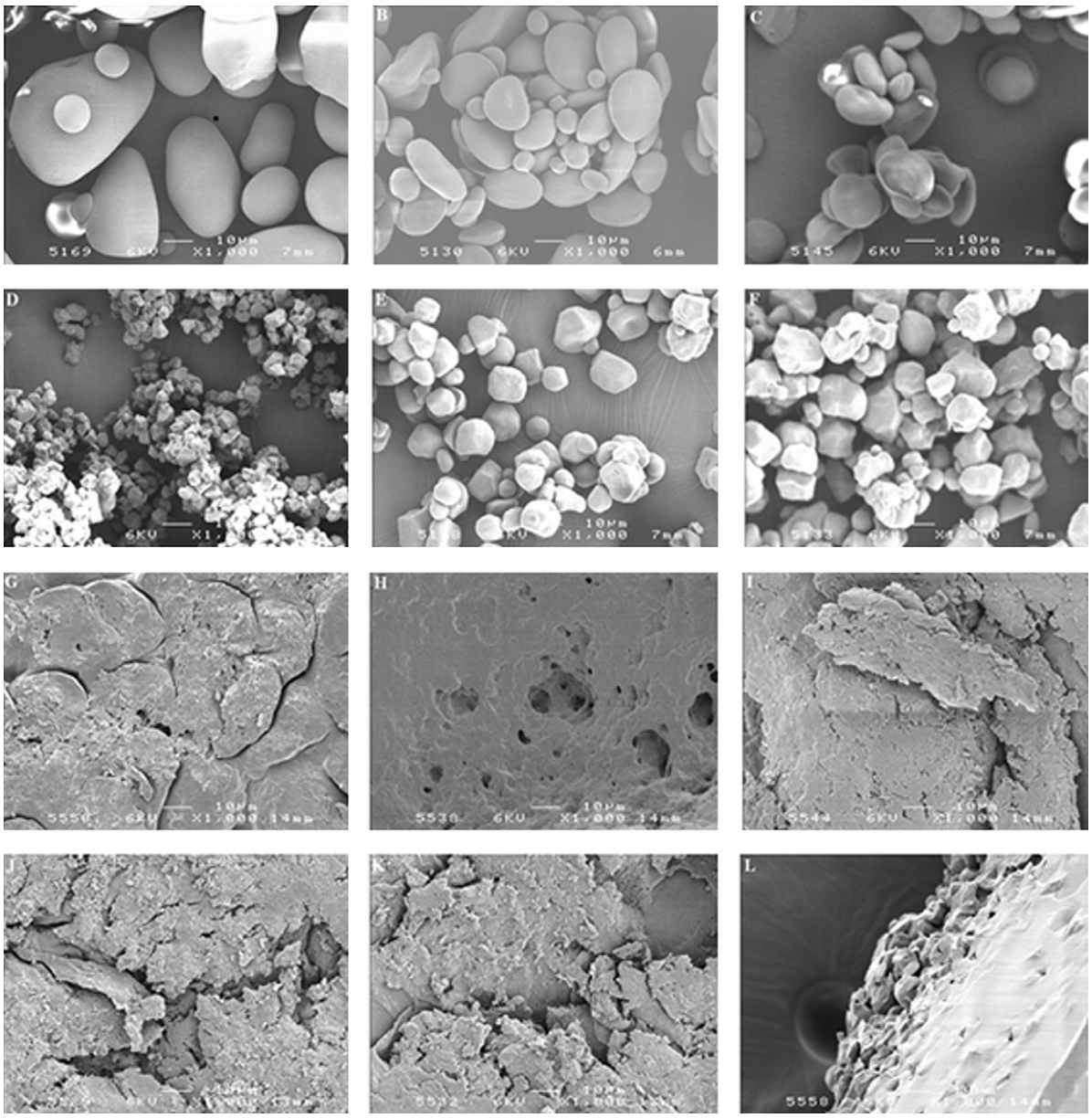

Fig. 4. The scanning electron micrographs of native starch species: potato (A), wheat (B), barley (C), rice (D), corn (E) and waxy corn (F). And scanning electron micrographs of gelatinized starch species after dissolution in [BMIM]Cl: potato (G), wheat (H), barley (I), rice (J), corn (K) and waxy corn (L).

ionic liquid in 10 min and formed clear solutions that were not too viscous.

For comparison starch species were dissolved into IL at 80 °C by using a conventional heating. Starches formed very viscous gels and their mixing was not satisfactory. Cereal starches dissolved after 2 h heating but the potato starch dispersion still was slightly opaque after 8 h heating. The SEM images showed a clear difference in the morphology of the native potato starch (Fig. 5) depending on the heating method. The microwave heating totally disrupted the starch granules. However, granules of the conventionally heated potato starch were distinguishable. Among the granules there were starch chains separated from the granules. It is possible that [BMIM]Cl could also affect the surface gelatinization of the starch granules. It is known that concentrated chloride salts surface gelatinize starch granules even at room temperature (Pan & Jane, 2000) and that microwave energy affects on the water molecules present in the crystalline regions of starch granules and enhances the rupture of granules (Palav & Seetharaman, 2007).

3.6. Effect of heating on starch dispersions in [BMIM]Cl by utilizing conventional and microwave heating

Starch dispersions were heated at 100, 125 and 150 °C to compare their behaviour in [BMIM]Cl at different temperatures. The effect of the heating method, conventional or microwave heating, was studied as well. Heating times ranged from 1 to 4 h. Ethanol-precipitated and dried starches were analyzed with HPLC-ELSD. Results for the heat-dispersed barley, wheat and potato starch in [BMIM]Cl at various reaction conditions are presented in Table 2.

Barley and wheat starch were conventionally heated at 100 °C for 2 h. The chromatogram of the dispersed barley starch showed one peak at the retention time of 16.5 min. Amylopectin (R_t 10 min) was not detected. The study of the wheat starch dispersion indicated that both amylopectin and amylose had slightly degraded and hence two fractions eluted at retention times of 12.5 and 16.5 min. The microwave-heated barley and wheat starch both resulted in one fraction (R_t 16.5 min) after 2 h heating. It was concluded that amylopectin mainly degraded but amylose much less.

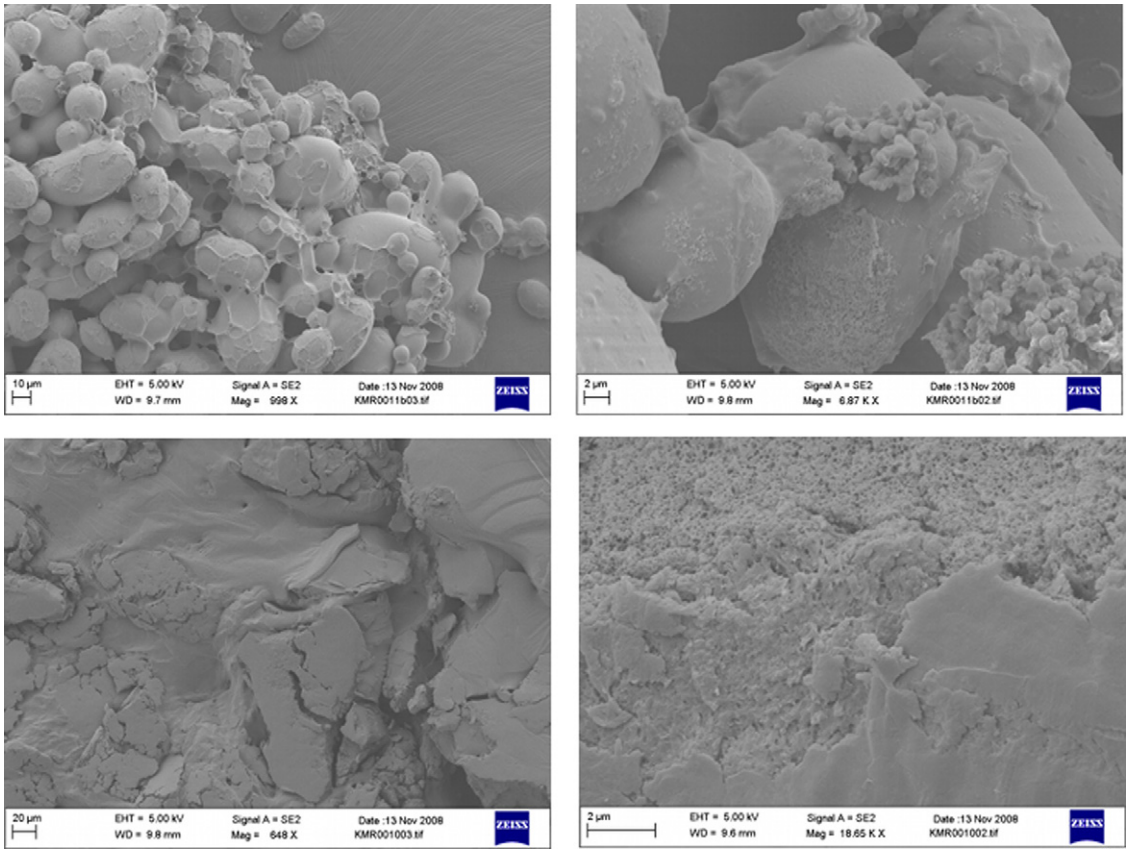

Fig. 5. The scanning electron micrographs of 8 h conventionally (top) and 2 h microwave (bottom) heated native potato starch.

Estimated molecular masses of degraded polymers were roughly 25 kDa. After the heating [BMIM]Cl was pale yellow. The HPLC chromatograms of various reactions with barley starch are provided as a Supplementary material.

The temperature was increased to 150 °C and the dispersions of barley and wheat starch were conventionally heated for 2 hours. The HPLC-ELSD chromatograms showed one peak (R_t 19 min) corresponding to polymers <5 kDa. The brown colour of the ionic liquid referred to a formation of small sugars during the heating. It has been reported that a high temperature causes caramellization (Izydorczyk, 2005), and that a controlled decomposition of hexoses yields furan derivatives (Leihmann & Rapp, 1994). 1-Ethyl-3-methylimidazolium chloride is reported to convert glucose to 5-hydroxymethylfurfural, mannose and 1,6-anhydroglucose at 180 °C (Zhao, Holladay, Brown, & Zhang, 2007). No such components were detected in our EtOH-precipitated product.

Table 2
Effect of the heating method, temperature and time on the degradation of some starch species dispersed into [BMIM]Cl.

Starch	Heating method	T (°C)	t (h)	R_t (min)	M_w (kD)
Barley	Oil bath	100	2	16.5	ca. 25
Barley	Oil bath	125	2	17	>5
Barley	Oil bath	150	2	19	<5
Wheat	Oil bath	100	2	12.5 and 16.5	>1400 and ca. 25
Wheat	Oil bath	125	2	17	>5
Wheat	Oil bath	150	2	19	<5
Potato	Oil bath	125	2	11.5 and 16	>1400 and ca. 25
Potato	Oil bath	125	4	13 and 17	>1400 and >5
Cereal[a]	MW	100	2	16.5	5–25
Cereal[a]	MW	125	1	17	>5
Cereal[a]	MW	125	2	19	<5
Cereal[a]	MW	125	4	20	1–5
Cereal[a]	MW	150	1	21	ca. 1
Potato	MW	125	1	13 and 17.5	>1400 and >5
Potato	MW	125	2	13.5 and 18.5	>1400 and <5
Potato	MW	125	4	14 and 20	>1400 and 1–5

[a] Wheat, barley, rice and corn.

The microwave-heated barley and wheat starch yielded one ca. 1 kDa-sized polymer fraction (R_t 21 min) after 1 h heating at 150 °C. The ionic liquid turned to dark brown and the amount of precipitate collected after the ethanol treatment was smaller than in previous experiments indicating more excessive decomposition of sugars.

The temperature was decreased to 125 °C and each of six starch species were studied after heating of 2 and 4 h. After 2 h conventional heating precipitated cereal starches, including waxy corn, eluted in one fraction (R_t 17 min). The HPLC chromatogram of conventionally heated barley starch is shown in a Fig. 6A. Heating of 2 more hours did not significantly change the outcome. Molecular masses of the yielded polymers were >5 kDa. Potato starch showed a different behaviour and eluted in two fractions (R_t 11.5 and 16 min) after 2 h heating (Fig. 6B). Amylopectin of the potato starch, but not cereal starches, contains phosphomonoesters. The first fraction possibly contained negatively charged phosphomonoesters of amylopectin molecules with positively charged imidazolium ring of the ionic liquid (Chaumont, Schurhammer, & Wipff, 2005). According to the retention time the second fraction contained the slightly degraded amylose. After 4 h heating and the usual work-up the product still eluted in two fractions (R_t 13 and 17 min). The study indicated that the potato starch with large granules dispersed and degraded slower than the cereal starches. Phosphomonoesters of potato starch possibly hindered depolymerization of amylopectin and therefore depolymerization of amylose was observed simultaneously. Ionic liquid was pale orange after the heatings.

Similar experiments were performed by using microwave heating at 125 °C. Each cereal starch was heated for 1, 2 and 4 h and

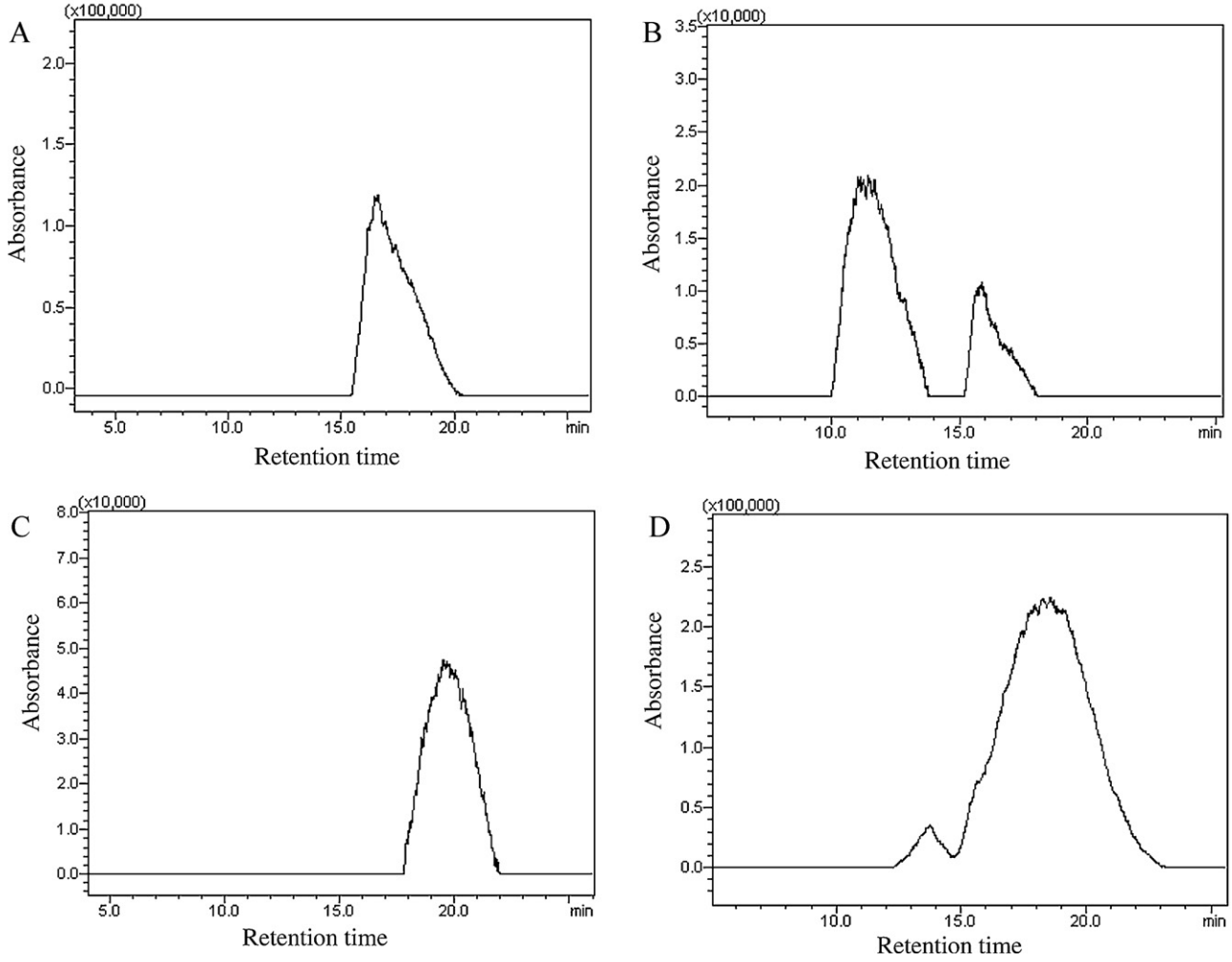

Fig. 6. The HPLC-chromatograms of heat-dispersed barley starch (2 h) in [BMIM]Cl at 125 °C after conventional heating (A) and microwave heating (C). The HPLC-chromatograms of heat-dispersed potato starch (2 h) in [BMIM]Cl at 125 °C after conventional heating (B) and microwave heating (D).

analyzed by HPLC-ELSD. Their chromatograms each contained one product peak at the retention time of 17, 19 and 20 min, respectively. The chromatogram of barley starch after 2 h heating is shown in Fig. 6C. After 4 h heating all cereal starches degraded to polymers <5 kDa. Again the potato starch eluted in two fractions at the retention times of 13 and 17.5 min, 13.5 and 18.5 min, 14 and 20 min after 1, 2 and 4 hours, respectively (see Supplementary material). The chromatogram of potato starch after 2 h heating is shown in Fig. 6D. The first fraction contained products with molecular masses higher than 1.4 MDa and the second fraction 1–5 kDa-sized polymers. The colour of the ionic liquid darkened as the heating time increased.

These studies indicated that the heating of the starch species in [BMIM]Cl led to the depolymerization of starch to smaller polysaccharides. Acid hydrolysis of starch is known to occur in two steps (Daniel & Whistler, 1994). Amorphous regions, rich in amylopectin and (1,6)-α-D-branching hydrolyze first. In crystalline regions of a starch granule hydrolysis occurs slowly. We suggest that depolymerization of starch in [BMIM]Cl might proceed by the similar manner. When starch dispersion in IL is heated it is assumed that [BMIM]Cl more easily penetrates into amorphous region than into crystalline regions and hence degradation of amylopectin is observed prior to amylose. Heating especially with microwaves increases the kinetic energy of the polar and ionic compounds and enhances the solubility of starch into IL. Even if starch species are dried prior to the reactions there is always some water present in it. We assume that this water is available in these reactions. Dried [BMIM]Cl also contain a trace amount of water. Therefore also some HCl might be present in reaction medium which can promote depolymerization of the starch. A selective degradation of amylose to monomeric products was not detected in the course of this study as was stated in the patent (Myllymäki & Aksela, 2005).

4. Conclusions

The degradation of six native starches (wheat, barley, potato, rice, corn and waxy corn) in [BMIM]Cl was studied by a high-performance liquid chromatography equipped with an evaporative light scattering detector (HPLC-ELSD). The developed HPLC-ELSD method enabled a detection of large biomolecules, amylopectin and amylose, as well as small sugars such as glucose at the same time. Starches were heat-dispersed into ionic liquid media under controlled microwave conditions or in oil bath. The effect of the temperature, heating time and method on starch components, amylose and amylopectin, as well as polysaccharides, that were generated during the heating, were studied.

Conventionally heated native starch dispersions in [BMIM]Cl formed transparent solutions at 100 °C and under microwave conditions already at 80 °C. The dissolution time was characteristic for each starch species. The SEM images of the gelatinized starches showed that corn, rice, wheat and barley starches were fully gelatinized, but waxy corn and potato starch still contained some granules. Amylopectin and amylose fractions of

cereal starches yielded by DMSO or ionic liquid treatment were successfully detected by using the HPLC with the ELS detector, since both solvents destroyed the granular structure of the native starch. Retention times for amylopectin and amylose were different enough so that two separate peaks were seen in the chromatograms. In [BMIM]Cl the degradation of amylopectin was observed immediately after starch granules were dispersed into ionic liquid and later also amylose degraded. Amylopectin of potato starch degraded slower than that of cereal starches. Therefore, this ionic liquid media cannot be used for the solvation of the starch species. The molecular size of the product decreased with the increasing reaction temperature and time. The microwave heating speeded up dissolution of starch as well as its degradation compared to the conventional heating in oil bath.

It is assumed that hygroscopic nature of [BMIM]Cl together with the moisture present in starch or the humidity of air can form HCl, which could catalyze the depolymerization. Therefore, the depolymerization did not require a separate, additional acid catalyst.

The research considering of the starch hydrolysis by using separate acid in IL has been carried out and is reported in a near future.

Acknowledgements

The authors thank Academy of Finland (KETJU Program SusProc Project), and TEKES for the financial support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2010.12.011.

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