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Pressure-induced changes in the structure of corn starches with different amylose content

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Abstract

Hylon VII and waxy corn starch–water suspensions (30%) were subjected to high pressure treatment at 650 MPa for 3, 6 and 9 min. Cross-polarisation/magic angle spinning (CP/MAS) ^{13}C NMR, X-ray, HPLC, optical microscopy (LM) and scanning electron microscopy (SEM) were used to monitoring physico-chemical changes in the structure and microstructure of starch preparations. The NMR spectra of pressurised starches showed a displacement of the chemical shift associated to C1 atoms as well as changes in peak intensities for C1, C4 and C6 after 9 min of treatment. The treatment of starch suspension with high pressure resulted also in a significant decrease in degree of crystallinity (DC) along with time of treatment and, in the case of waxy corn starch resulted in complete loss of crystallinity already after 3 min of treatment. The profile of the molecular weight distribution of waxy corn starch pressurised for 6 and 9 min differed significantly from the one obtained for native starch. The microscopy analysis showed that the pressurisation of waxy corn after 3 min lead to formation of gel structure.

Keywords: Starch; High pressure; (CP/MAS) ^{13}C NMR; X-ray diffraction; HPLC; Microstructure

1. Introduction

It is generally believed that high pressure technology defined as 'mild technology' offers a new possibility for the application of starch to food products. The high pressure can evoke gelatinisation of starch granules in starch–water suspension already at room temperature (Stolt, Stoforos, Taoukis, & Autio, 1999). However, the pressure induced gelatinisation is significantly different from heat induced gelatinisation (Rubens & Heremans, 2000; Stute, Klingler, Bogusławski, Eshtiaghi, & Knorr, 1996; Stolt, Oinonen, & Autio, 2001; Stolt et al., 1999). The gelatinisation of starch granules during heating in excess of water was defined by Hermansson and Svegmark (1996) as phase transition of granules from an ordered state to a disordered one. This phenomenon was mostly related to the granules hydration, rapid swelling and advanced leakage of amylose that in turn resulted in loss of anisotropic order, crystallinity as well as granular shape. On the contrary, most of the starches subjected to high pressure (600 MPa), in excess of water retain a granular shape demonstrating limited power of swelling and amylose release (Douzals, Perrier-Cornet, Gervais, & Coquille 1998; Stute et al., 1996). Also, Stolt et al. (1999) reported that the rheological properties of waxy corn starch suspension (10%) after high pressure treatment (450–600 MPa) differed significantly from these ones after hydro-thermal treatment. The observation made under polarised light by Stute et al. (1996) indicated that pressurised wheat and corn starches (25% suspension) started to progressively lose birefringence at a pressure of 450 MPa and after 15 and 30 min of treatment. Stolt et al. (2001) showed that the birefringence of barley starch suspension (25%) was immediately lost after its treatment at 550 MPa, although the starch granules retained the granular shape. Whereas, the potato starch suspension (5%) subjected to high pressure treatment at 600 or 800 MPa for 20 min. demonstrated damaged structure of granules and lost of birefringence under optical microscopy

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(Gomes, Clark, & Ledward, 1998). Błaszczak, Valverde, and Fornal (in press) showed significant changes in the structure of potato starch granules already after 3 min of treatment at 600 MPa in excess of water (10% suspension in water). By using SEM, these authors observed that the high pressure-treated starches demonstrated two distinctly differentiated zones. The outer zone of granules remained unchanged and it corresponded to the more organised part of granule, whereas the interior part of granules was completely destroyed and formed gel-like structures. The observation by SEM also confirmed by (CP/MAS) ^{13}C NMR studies on pressurised potato starch conducted by Błaszczak and co-authors seemed to be closely related to the previous suggestion of Rubens and Heremans (2000) that the changes in the internal granule structure evoked by high pressure may be resulted from the hydration and/or melting of the crystalline structures. The phenomenon of birefringence loss by the pressurised starch granules may be resulted from the disruption of a radial orientation of the crystallites that, according to Yuryev, Wassermen, Andreev, and Tolstoguzov (2004), are predominantly formed by the amylopectin chains in the double helical form.

It is well known that solid state NMR is widely used for characterising some degrees of molecular order (i.e. helicity) in the structure of starchy substrates (Gidley & Bociek, 1985) and for the identification of the crystalline order and amorphous sites within structure of native starches as well as after their different treatment (Bogracheva, Wang, & Hedley, 2001; Paris, Bizot, Emery, Buzare, & Buleon, 1999; Tamaki, Hisamatsu, Teranishi, Adachi, & Yamada, 1998; Waigh, Gidley, Komanshek, & Donald, 2000). It was shown that the C1 and C4 carbons sites were involved in glycosidic linkage (Gidley & Bociek, 1985). The resonances of C1 atoms with distinct multiplicities, different for A- and B-type polymorph, have been assigned to the crystalline areas in starch structure. Whereas, the changes in C1 multiplicity and/or chemical shift of the C1 carbons as well as resonances of C4 atoms have been correlated to the amorphous sites within starch structure (Bogracheva et al., 2001).

The aim of this study was to investigate the changes in the physico-chemical structure and microstructure of starches with different amylose content (obtained from Hylon VII and waxy corn starch) which were subjected to high (hydrostatic) pressure treatment in excess of water as a function of time.

2. Material and methods

2.1. Material

The experimental material was Hylon VII, refined from high amylose (68%) maize that was a gift of National Starch and Chemical, Food Starch, Poland. The second raw material—the waxy corn starch (trace amounts of amylose) was purchased from Sigma (S-9679).

2.2. Sample preparation

The pressure treatment was performed in excess of water, i.e. using 30% (w/w) starch–water suspension. The suspension was closed into Teflon tubs (10 mL), precisely mixed, deaerated, closely sealed and pressure-treated.

2.3. Pressure treatment

The pressure treatment of starch–water suspensions was performed according to the method of Błaszczak et al. (in press) using a high pressure device (LV30/16, The Centre of High Pressure Analysis, Polish Academy of Sciences, Warsaw, Poland). The Teflon tubes were put into a high pressure chamber (with the capacity of approximately 25 cm^3) filled with pressure transmitting medium which also minimised adiabatic heating. The samples were pressure-treated at the level of 650 MPa for 3 and 9 min at a rate of approximately 10 MPa/s^{-1}. The temperature inside the pressure chamber averaged 20 ± 2 °C. The pressure treatment was performed in two repetitions for each combination.

After the pressure treatment, the starch 'preparations' were frozen rapidly in liquid nitrogen and freeze-dried. The obtained starch samples were analysed by HPLC, (CP/MAS) ^{13}C NMR, X-ray, LM and SEM microscopy.

2.4. HPLC

The starch samples (20 mg) were dissolved in 2 mL of a solvent mixture: dimethyl sulfoxide (DMSO): water (9:1 v/v) by heating for 3 min at 100 °C. After filtration (0.4 μm), the samples were sonicated and subjected to high performance column PL gel 5 μm MIXED-C (300 × 7.5 mm) (Polymer Laboratories). A system consisting of two columns with the characteristics described above were combined and heated at 70 °C in heater column temperature controller (Waters) was used. The flow rate of the eluent (DMSO: water, 9:1 v/v) was equal to 1 mL/ min. The maximum pressure inside column was retaining at the volume of 3000 psi (HPLC pump Waters 510). The obtained fractions were analysed using a Differential Refractometer (Waters 410).

Molecular mass (up to 788,000) was determined by using a polysaccharide standard kit SAC-10 purchased from Polymer Laboratories

2.5. (CP/MAS) ^{13}C NMR spectroscopy

The NMR experiments were performed in a Bruker Avance™ 400 spectrometer (Bruker Analytik GmbH, Karlsruhe, Germany) equipped with a Bruker Ultrashield™ 9.4-T (^{13}C resonance frequency of 100.62 MHz), 8.9-cm

vertical-bore superconducting magnet. The CP/MAS NMR spectra were acquired at ambient temperature by using a standard Bruker broad-band MAS probe. Powdered samples were packed in a 4-mm zirconia rotors, sealed with Kel-F™ caps and spun at 5 kHz. ^{13}C-MAS NMR spectra with 1 ms CP contact time, 5 s recycle delay and high-power proton decoupling were acquired. Each free induction decay was subjected to standard Fourier transformation and phasing. The chemical shifts were externally referenced to the solid adamantane peak at 38.5 ppm. A total of 400 scans were averaged for each spectrum.

2.6. X-ray

The X-ray analysis was performed using a diffractometer type TUR 62 (Carl Zeiss, Germany) under the following conditions: X-ray tube Cu Kα (Ni filter), 30 kV, current 15 mA, scanning from $\theta = 2$ to 18°.

To avoid the influence of relative humidity on relative crystallinity, the starch samples were placed in desiccator and conditioned in the atmosphere of relative humidity of 92% for 48 h. To this end the desiccator was filled with sodium carbonate saturated aqueous solution.

The changes in relative crystallinity between native and treated starches were expressed through the mathematical calculation of peak area under the curve in X-ray diffraction patterns of the analysed starches using Micro Image 4.0 for Windows (Olympus Optical co. Europe) (Błaszczak et al., 2003).

2.7. Microscopy analysis

Changes in the starch microstructure resulting from high pressure and time of treatment were visualised by using: (i) optical microscopy (LM) technique: polarised light and (ii) scanning electron microscopy (SEM).

The analysed starch powders were suspended in a drop of water and covered by cover slip. The preparations were viewed and photographed in a LM microscope OLYMPUS BX60.

The starch powders for SEM analysis were stick on a specimen holder using a silver plate, and then coated with gold in a vacuum evaporator (JEE 400, Jeol). The obtained specimens were viewed in a JEOL JSM 520 scanning electron microscope at the accelerating voltage of 10 kV.

3. Results and discussion

Since the most significant changes in starch structure were observed in the (CP/MAS) ^{13}C NMR patterns, HPLC profiles and microscopy pictures of samples treated at high pressure during 9 min, only figures corresponding to these results as well as to those of native starches were selected to be included in this work.

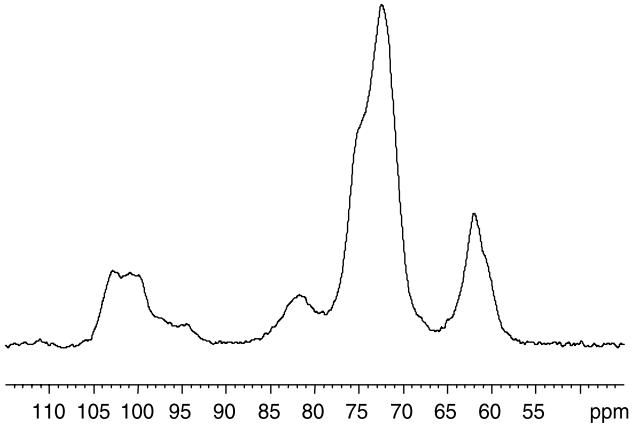

Fig. 1. (CP/MAS) ^{13}C NMR spectra of native Hylon VII.

3.1. (CP/MAS) ^{13}C NMR analysis

The (CP/MAS) ^{13}C NMR patterns for native Hylon VII and native waxy corn starch are presented in Figs. 1 and 2.

In the spectrum of Hylon VII (Fig. 1), different resonances and multiplicities for C1 glucose atom at the 94–105 ppm region were observed. The C1 multiplicities with similar signals intensities were found at 100–102 ppm. According to Paris et al. (1999), these regularly arranged conformations of crystalline packed chains resulting in narrow signal frequency C1 bands with multiplicities. Bogracheva et al. (2001) also observed the C1 multiplicities as double peak at 99–100 ppm region in the pattern of potato starch. These authors claimed that the resonances appearing in that region as C1 double peak are typical for the ordered parts of starch with B-type crystallites.

The spectrum recorded for native waxy corn starch (Fig. 2) showed different features to that of Hylon VII regarding the resonances for C1 carbon, especially. The signal resonance for C1 at 94–105 ppm region was also observed. However, the C1 multiplicities for ordered parts of starch with A-type crystallites was found to be a triplet at 99–102 ppm region. Gidley and Bociek (1985) found that a crystalline debranched glycogen and the second one

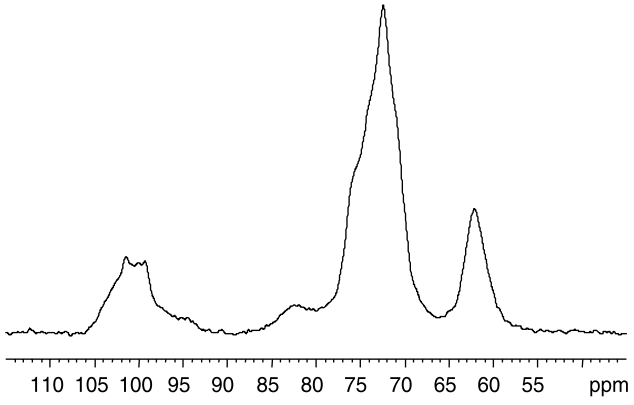

Fig. 2. (CP/MAS) ^{13}C NMR spectra of native waxy corn starch.

obtained from low molecular weight branched (1–4)-α-D-glucan produced by acid degradation of waxy maize showed three peaks at similar spectral positions (100.4, 99.2 and 98.2 ppm). However, the crystalline material produced by acid degradation of waxy maize starch demonstrated additionally extra signal intensity at 102–105, 94–98 and 81–83 ppm. Since, the disruptive influence of branch points on the packing of ordered structures were suggested by Gidley and Bociek (1985), these authors assigned these resonances to the amorphous sites within starch structure. In the spectrum of waxy corn starch as well as in that one of Hylon VII, the resonances at similar regions were also observed. The resonances at 80–84 ppm were assigned to C4 carbon. The C4 peak was strongly pronounced in the spectrum of Hylon VII, whereas in the spectrum of waxy corn starch it exhibited very low-intensity. It is well known that the resonances at 102–105 and 81–83 ppm correspond to the amorphous regions within starch structure (Bogracheva et al., 2001; Gidley & Bociek, 1985), that according to Paris et al. (1999) resulted from amorphous single helical conformations.

The other resonances related to C2, C3 and C5, and C6 carbons were observed in both spectra (native Hylon VII and waxy corn starch) at similar regions: 68–78 and 59–65 ppm, respectively. The high-intensity peak centred at 72 ppm demonstrated a low-field shoulder, more pronounced in the case of Hylon VII.

The (CP/MAS) ^{13}C NMR patterns obtained for the starches treated under high pressure condition at 650 MPa for 3 min did not show significant changes in their chemical structure compared to that of untreated starches (figures not presented). Whereas, an increase in the time of treatment (650 MPa) to 9 min evoked changes in chemical structure of both starches (Hylon VII and waxy corn starch).

The (CP/MAS) ^{13}C NMR pattern for pressurised Hylon VII and waxy corn starch (650 MPa/ 9 min) (Figs. 3 and 4) showed changes mostly in the signal resonances for C1 glucose carbon. Instead of the C1 multiplicities typical for starch with B-crystallites, a single broad peak corresponding

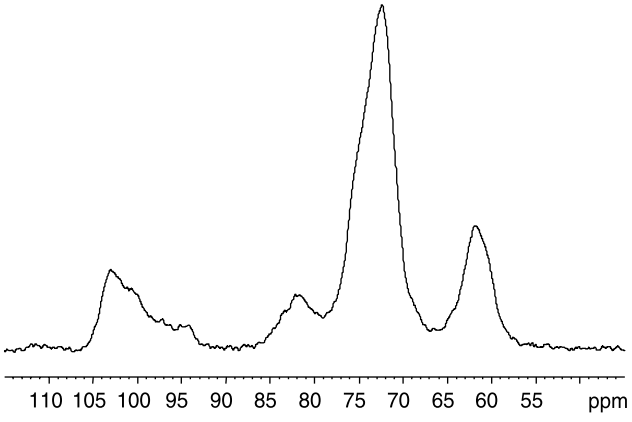

Fig. 3. (CP/MAS) ^{13}C NMR spectra of high pressure-treated Hylon VII at 650 MPa for 9 min.

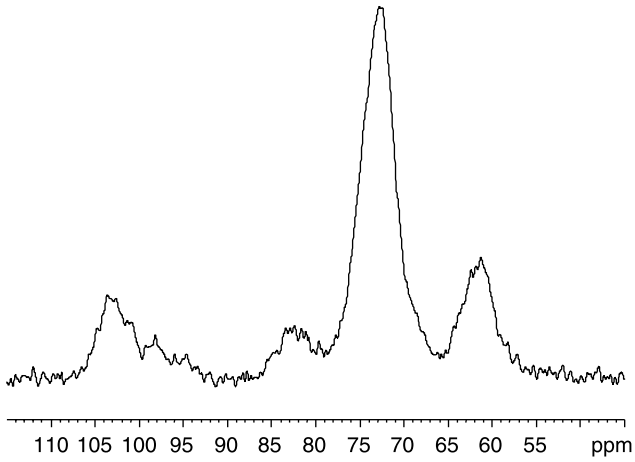

Fig. 4. (CP/MAS) ^{13}C NMR spectra of high pressure-treated waxy corn starch at 650 MPa for 9 min.

to the C1 glucose carbon, in the range 94–105 ppm of the spectrum of high pressure-treated Hylon VII, was observed. The C1 peak seemed to be centred around 103 ppm (a high intensity band) and it showed a slight shoulder at around 101, 98 and 94 ppm. Interestingly, in the spectrum of pressure-treated (650 MPa/9 min) waxy corn starch (Fig. 4) the triplet for C1 carbon at 98–102 ppm was not observed. The C1 resonances of high pressure treated waxy corn starch at 98–105 ppm region were found and showed at least four components at 103, 101, 98 and 94 ppm. Tamaki et al. (1998) also observed a distinct disappearance of the C1 crystalline multiplet and an increase of the peak intensity at the 103 ppm region in the (CP/ MAS) ^{13}C NMR spectra of maize starches (with different amylose content) after their mechanical treatment (ball-milling). The spectra of treated starches, presented by Tamaki and co-authors showed similar features to those shown in this work. From this comparison, it may be assumed that pressurised starches (Hylon VII and waxy corn starch) showed an amorphous character—especially taking into consideration the changes which appeared in the C1 region. According to Paris, Bizot, Emery, Buzare, and Buleon (2001), the changes in C1 region, observed by Tamaki and co-authors, may result from a conversion of double to single helices or to a distribution of dihedral angles with values similar to those found in single helices. These authors performed a spectral decomposition of C1 peaks in the spectra of various starch substrates prepared by different methods (casting, freeze drying, solvent exchange). The decomposition of C1 resonances allowed these authors to reveal the presence of some C1 components (at 103.2–103.4, 102.9, 100–101.4, 97.0–98.6 and 94.4–94.5 ppm region) that were assigned to the main different populations of α (1–4) linkages. Following this authors' work, the signals at 103 ppm may be assigned to an angular conformation similar to single helices present in Va (anhydrous) type of structure. According to Paris et al., 2001, the resonances observed at 101 ppm correspond to a double helical conformation. Whereas the ones at 98 ppm

may be attributed to the α (1–6) linkages or resulted from different conformations of chains within the branched regions (Morgan, Furneaux, & Larsen, 1995). According to Paris and co-authors, the resonances at 94 ppm correspond to the conformations that result from the drastic method of treatment (i.e. freeze-drying). As it was mentioned above, the peaks at 103, 101 and 98 ppm were strongly pronounced in the spectra of pressurised starches. On the contrary, the resonances at 94 ppm demonstrated low-intensities. At high freezing rates (applied during starch sample preparation), the effect of concentration dominates the effect of ice crystal formation (Heertje, Leunis, van Zeijl, unpublished data), thus the changes occurring in chemical structure of the starches obtained were minimised. The higher signal intensities at 94 ppm, observed by Paris and co-authors, probably indicates that an isolated starch fraction (amylose) is much more susceptible to freeze-drying than complex system of both polymers—amylose and amylopectin. As Heertje and co-authors reported, starch demonstrates cryoprotective action because of polysaccharide of which they are composed. Based on the results obtained here as well as on those reported in the literature cited above, it can be stated that the amorphous character of the spectra of pressurised starches resulted mainly from high pressure treatment rather than from freeze-drying. It was showed that the high pressure may evoked polymer disordering and an increase in the number of conformations (Błaszczak et al., in press) what, to high extent, may result from a reversible hydration of the amorphous phase followed by an irreversible distortion of the crystalline region in granule structure (Rubens & Heremans, 2000). The resonances corresponding to C2–C6 carbons observed in the spectra of pressurised starches were characterised by lower signal intensities compared to those ones shown in the spectra of native starches. Only in the case of the spectrum of pressurised waxy corn starch, an increase in the signal intensity of C4 (at 83 ppm) resonances was found. According to Gidley and Bociek (1985), the changes in the spectral position and intensity of C1 and C4 carbon peaks result from the fact that these two carbons are involved in glycosidic bonds and they seem to be the most sensitive for polysaccharide conformation. The resonances at 82 ppm corresponded to C4 demonstrating V type single helical conformation which dominates in the amorphous state (Morgan et al., 1995; Paris et al., 2001).

3.2. X-ray analysis

The diffraction pattern of native Hylon VII (Fig. 5a) apart from the B-type crystallinity also demonstrates an additional broad peaks at 12–13 and 19–20°. Similar pattern of high amylose corn starch was obtained by Shamai, Bianco-Peled, and Shimoni (2003). These authors assigned an additional peak (appeared at 19.9°) to V-type polymorph. According to Yuryev et al. (2002) as well as Gernat, Radosta, Anger, and Damaschun (1993), the V-polymorphic form may be

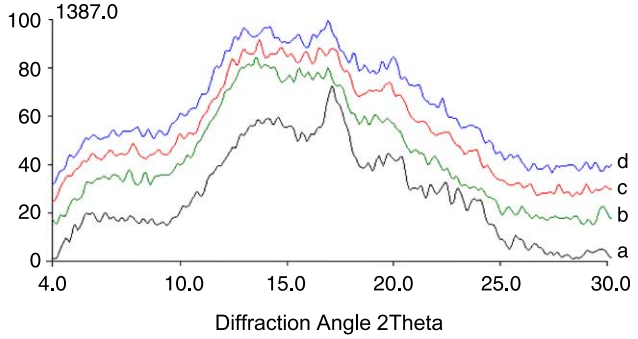

Fig. 5. X-ray diffraction patterns of (a) native Hylon VII and treated one with high pressure at 650 MPa after, (b) 3 min, (c) 6 min and (d) 9 min.

assigned to the closely packed single helices of amylose–lipid complex in high amylose native starch granules. The high amylose starches beside B-type may contain Vh-type that is a crystalline structure of fatty acid-amylose complexes being formed by single helices with six anhydroglucose monomer residues per helical turn (Gernat et al. 1993; Yuryev et al., 2002). Paris et al. (2001) reported also that the Vh-type polymorph may result from co-crystallisation of amylose as single helices, i.e. with fatty acids. Also Gernat et al. (1993) found the presence of B- and Vh-type crystals in the diffraction patterns of maize starches containing at least 50% of amylose. The same conclusion was obtained during DSC investigations of high amylose maize and pea starches (Kozhevnikov et al., 2001; Matveev et al., 2001).

The diffraction patterns of native waxy cornstarch, as was expected, showed A-type crystallinity (Fig. 6a) (Fornal, Błaszczak, & Lewandowicz, 1998).

The digital data showed that the degree of crystallinity calculated (DC) for the native Hylon VII and waxy corn starch averaged 17 and 43%, respectively. These calculations seem to be related to the results presented by Matveev et al. (2001). These authors as well as Gernat et al. (1993) demonstrated that the overall DC of corn starches decreased along with an increase in amylose content. According to Matveev and co-authors and Gernat et al. (1993), the waxy corn starches (1–5% amylose) demonstrated DC between 31 and 40%, whereas most of the high amylose starches (50–70% amylose) between 14 and 17%.

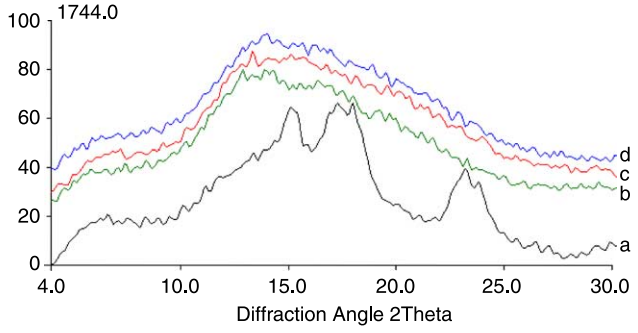

Fig. 6. X-ray diffraction patterns of (a) native waxy corn starch and treated one with high pressure at 650 MPa after, (b) 3 min, (c) 6 min and (d) 9 min.

The Hylon VII subjected to high pressure for 3, 6 and 9 min demonstrated a progressive decrease in the degree of crystallinity, i.e. from 17 to 14, 11, and 9%, respectively. Despite these changes, the high pressure-treated Hylon VII (for 9 min) retained, at least partially, a crystalline structure (Fig. 5b–d). On the contrary, the waxy corn starch (Fig. 6b–d) demonstrated an amorphous character already after 3 min of treatment. These data showed that the volume of pressure of 650 MPa was not enough to evoke a complete destruction of crystalline structure of Hylon VII. Only both factors used here, i.e. pressure action and the prolonged time of treatment, led to a significant changes in the structure of Hylon VII.

These results distinctly confirmed the (CP/MAS) ^{13}C NMR analysis indicating that the changes in starch structure resulted from high pressure and time of treatment and they were related mainly to the destruction of the crystalline part of the granule. It is well known that at least three main structural elements form the granules: amorphous, amorphous lamellae alternating with crystalline lamellae and crystalline lamellae (A- or B-crystallites) (Yuryev et al., 2002). The changes in C1 region observed in NMR spectra as well as distinct decrease in crystallinity observed in the diffraction patterns of pressurised starches showed that the high pressure affected mainly the destruction of the crystalline parts of the granule. However, these results also showed that Hylon VII exhibited lower susceptibility to pressurisation than waxy corn starch. Stute et al. (1996) reported that A- and C-type starches were more susceptible to high hydrostatic pressure (600 MPa) than B-type ones. However, they demonstrated that rule could not be confirmed since the starch analysed by them ‘amylocorn’ (B-type polymorph) was more sensitive to treatment at 600 MPa than taro-starch (A-type polymorph). It is necessary to mention that these suggestions were based mainly on microscopic analysis under polarised light.

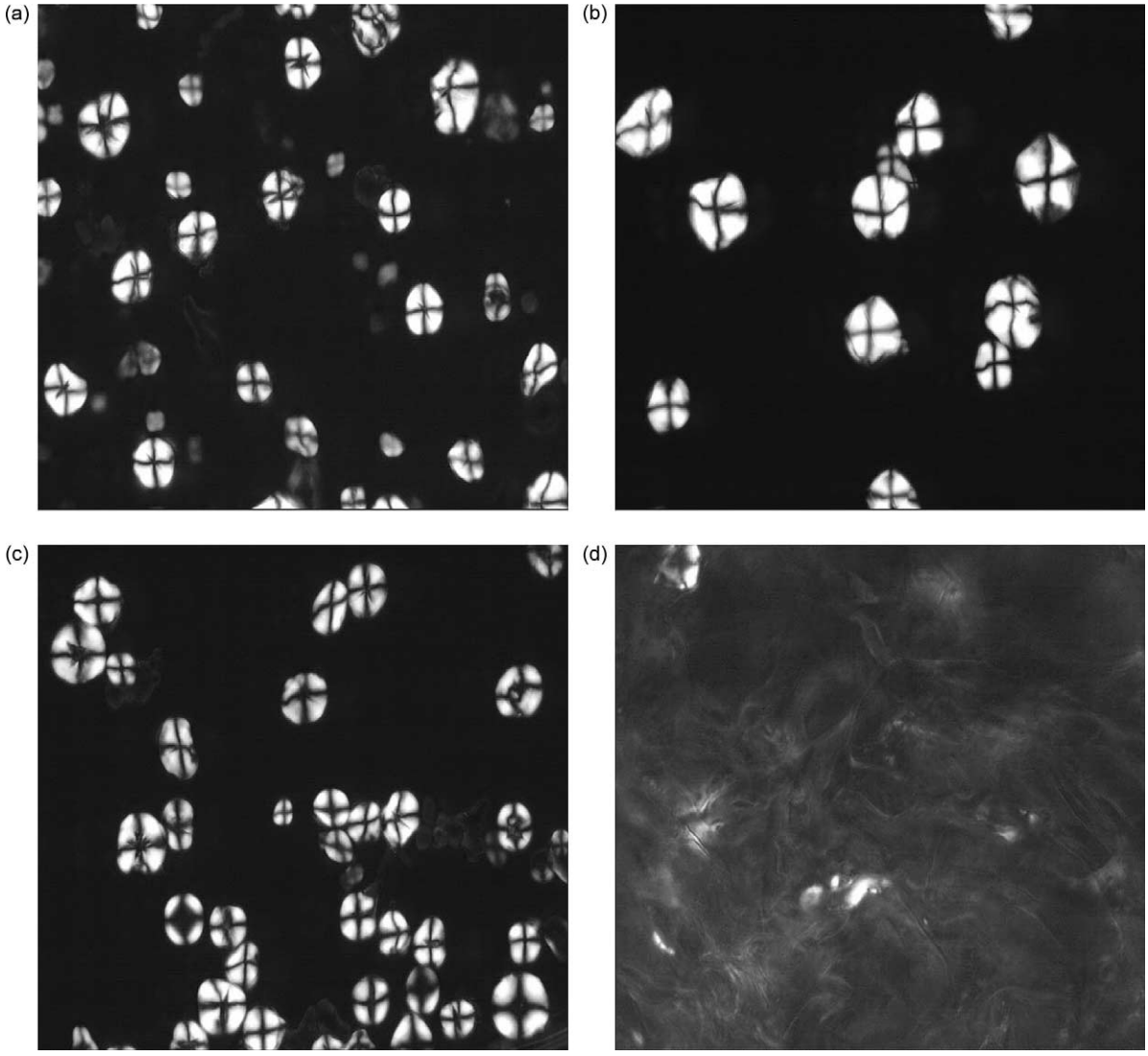

Fig. 7. Microstructure of starches in polarised light: native Hylon VII (a) and waxy corn starch (b), Hylon VII (c) and waxy cornstarch (d) treated with high pressure at 650 MPa for 9 min.

3.3. Microscopy analysis

The native Hylon VII and waxy corn starch demonstrated the typical birefringence under polarised light observed for native granules (Fig. 7a and b). The treatment with high pressure of Hylon VII for 3 min did not affect appearance in microscopy pictures of starch granules (figure not presented). Whereas, the starch granules of Hylon VII treated for 6 (figure not presented) and 9 min at 650 MPa only in part demonstrated the effect of Maltese cross (Fig. 7c). The granules had a hole-like appearance inside and only the outer part of starch structure still retained the birefringence.

As expected, the waxy corn starch lost the birefringence already after 3 min of treatment with high pressure (figure not presented). Whereas the treatment of waxy corn starch for 6 (figure not presented) and 9 min (Fig. 7d) resulted in partial and complete loss of granular structure, respectively.

Stolt et al. (2001) using polarised light also found that the majority of granules of the barley (A-type) starch lost birefringence after treatment with high pressure at 450 MPa and 30 °C for 15 min. Stute et al. (1996) analysed high amylose corn starch 'amylocorn' and demonstrated partial loss of birefringence after high pressure treatment (600 MPa) too, whereas other B-type starches, i.e. potato, showed a Maltese cross in polarised light. The results obtained suggest that the changes observed in the structure of Hylon VII treated for 9 min and waxy corn starch resulted, most likely, from the disruption of a double helical form of the amylopectin chains. The crystallites, formed by these structures, indicated a radial orientation within the starch structure and are responsible for the effect of Maltese cross under polarised light (Yuriev et al., 2002).

The pictures obtained by scanning electron microscopy (SEM) of native Hylon VII and waxy corn starch showed the typical polygonal shape of corn starch granules with sizes ranged from 5 to 20 and 2 to 30 μm (longer axis), respectively (Fig. 8a and b). Among of the typical shape of granules of Hylon VII some longitudinal structures were also observed. Due to fact that these structures with iodine stained dark blue under LM, they were assigned to starch granule deformed during some technological processes. It is well know that some technological process may affect the granular structure of starch resulting in its deformation, i.e. spray-drying.

The detailed analysis under SEM of Hylon VII after treatment for 9 min. (Fig. 8c) seemed to explain the reason of lack of the granules ability to birefringence under

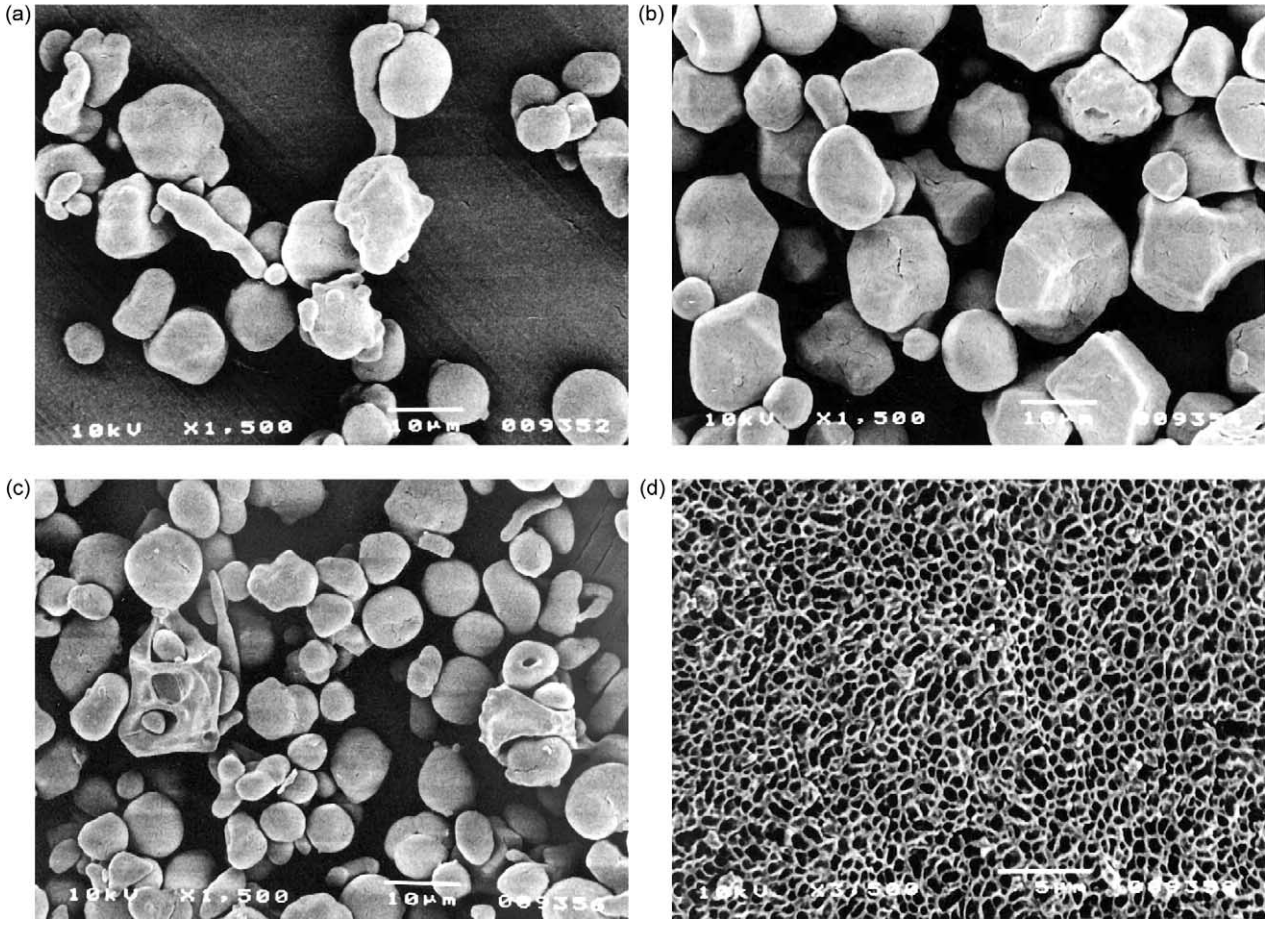

Fig. 8. SEM microstructure of starches: native Hylon VII (a) and waxy corn starch (b), Hylon VII (c) and waxy cornstarch (d) treated with high pressure at 650 MPa for 9 min.

polarised light. While the majority of granules retained granular shape, many of them appeared collapsed, deformed and demonstrated the hollow appearance. This phenomenon in starch granule was already observed under polarised light. Although the changes in granules structure were significant, the leaching of amylose outside granules which appeared slightly swollen was not observed. Stolt et al. (2001) studied the microstructure of barley starch subjected to high pressure and time treatment at different conditions did not observed leaching of amylose in LM pictures either. Błaszczak et al. (in press) found that granules of potato starch treated at 600 MPa for 3 min. demonstrated (under SEM) two distinct parts: the outer part was formed by unchanged structure related to a more organised polymer structure and the internal one completely filled with a gel-like network.

The microscopy pictures of waxy corn starch treated for 9 min (Fig. 8d) showed a complete breakdown of granules. Fig. 8d demonstrating a molecular dispersion of waxy corn starch after treatment for 9 min. That homogenous structure of a gel network was formed by the amylopectin phase. These observations were similar to those made by Stute et al. (1996). These authors showed that the treatment of waxy corn starch in excess of water (5% suspension) at a pressure of 600 MPa for 15 min lead to a complete disintegration of starch granules.

The microscopy observations were in good agreement with the results obtained by the NMR and X-ray analysis, as well as with the Rubens and Heremans' (2000) suggestion referred above.

3.4. HPLC analysis

Fig. 9a and b illustrate the molecular weigh distributions of native Hylon VII and native waxy corn starch, respectively. The profile of Hylon VII consists of two peaks: the first peak with low intensity appeared at the same retention time as amylopectin (788 kDa), the second one with higher intensity was related to molecular weight of amylose (404 kDa) (Fig. 9a). The waxy corn starch showed only one peak with the retention time corresponding to molecular weight of amylopectin (Fig. 9b).

Changes in the molecular weight distribution of high pressure-treated Hylon VII (Fig. 10a) were not observed.

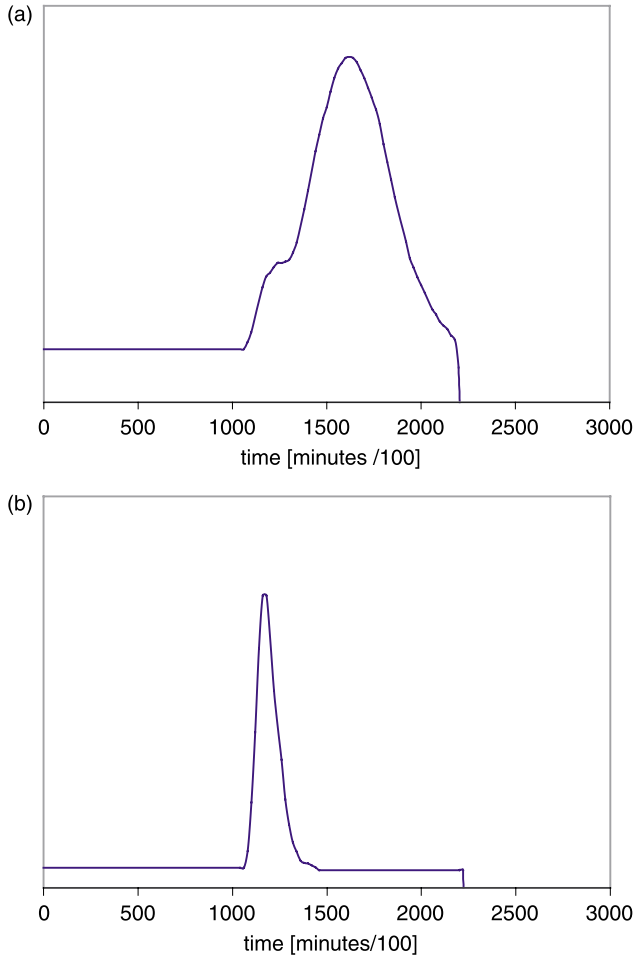

Fig. 9. HPLC profiles of molecular weight distribution of native (a) Hylon VII and (b) waxy corn starch.

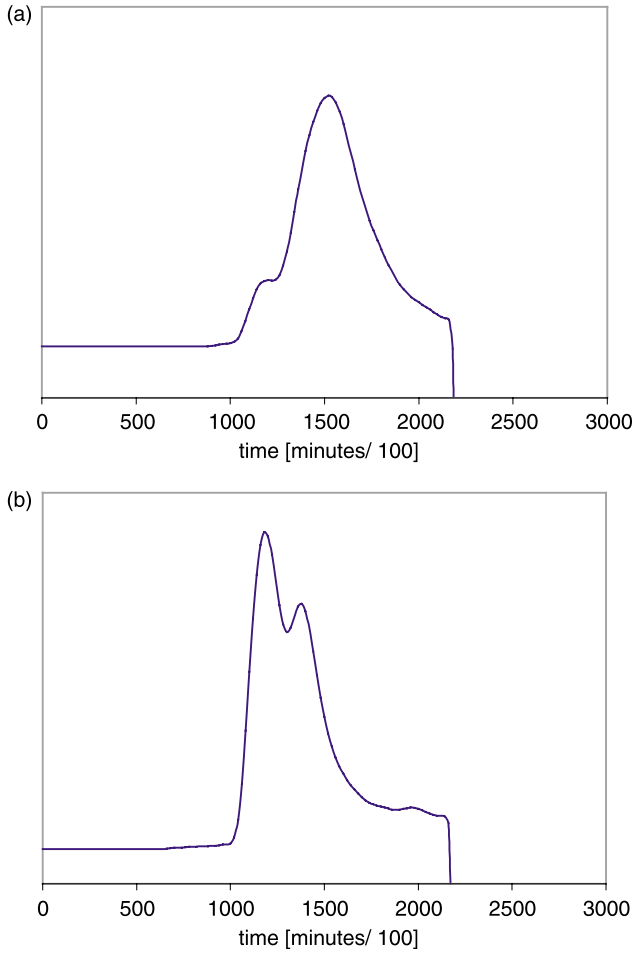

Fig. 10. HPLC profiles of molecular weight distribution of high pressure-treated (650 MPa for 9 min) (a) Hylon VII and (b) waxy corn starch.

Whereas, the HPLC profiles of waxy corn starch demonstrated changes in molecular weigh distribution after 6 (figure not presented) and 9 min of treatment (Fig. 10b). The HPLC profile of waxy corn starch treated for 6 min. showed one peak broader than that appeared at the same retention time of amylopectin. However, its maximum was slightly shifted towards to molecular weight lower than 788 KDa. The profile of the waxy corn starch pressurised for 9 min. indicated that the amylopectin formed a polydispersed product (Fig. 10b) with the retention time maximum shifted between the values related to the molecular weights of amylopectin and amylose, 788 and 404 kDa, respectively.

4. Conclusions

The high pressure treatment of Hylon VII and waxy corn starch suspension in excess of water evoked significant changes in the structure of the samples obtained that revealed the disruptive influence of high pressure on starch crystallinity. However, the results obtained showed different susceptibility of treated starches to high pressure. The waxy corn starch showed an amorphous character already after 3 min of treatment. Whereas a significant decrease in degree of crystallinity along with time of treatment was observed in case of pressure-treated Hylon VII. The (CP/MAS) ^{13}C NMR spectra of starches pressurised for 9 min demonstrated the most significant changes in C1 and C4 regions indicating amorphous sites within starch structure. Some changes in molecular weight distribution were also found for waxy corn starch treated above 6 minutes. Microscopy analysis (LM, SEM) showed that pressurisation of waxy corn starch resulted in a complete breakdown of the granules, whereas Hylon VII retained a granular structure after 9 min of treatment.

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