

EFFECT OF HIGH HYDROSTATIC PRESSURE ON THE STRUCTURE AND GELLING PROPERTIES OF AMYLOPECTIN STARCHES

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Waxy maize starch (APM), amylopectin wheat (APW) and amylopectin potato (APP) starches were subjected to high pressure treatment (650 MPa/9 min) in the excess of water. High sensitivity differential scanning microcalorimetry (HSDSC) and ¹H-NMR spectroscopy were used to analyse changes in the crystalline structure, gelling and osmotic properties of the pressurized starches. High pressure-treated APM and APW starches demonstrated almost complete gelatinisation of granules (GD=86% and GD=90%, respectively) and significant changes in their crystalline structure (melting of the A-type crystallites). Whereas, the degree of gelatinization of the “pressurised” APP starch accounted for 54%. The measurements of relaxation time constants (T_2) of water molecules in pressurized starch gels showed two different relaxations (46–720 ms and 8–120 ms). The complex relaxation of water molecules resulted directly from their different mobility that was triggered by differences in the structure of pressurized starch gels. These differences, *i.e.* ratio of swollen elements (granule remnants) to the non-swollen elements (crystalline, ordered structures), significantly affected not only the relaxation of water molecules in the gels but also determined their swelling index. The high pressure-treated starches after rehydration formed a gel structure that revealed low values of a swelling index.

INTRODUCTION

It is generally believed that amylopectin molecules demonstrate polymerization degree (DP) ranging from 3×10^5 to 3×10^6 [Van Hung *et al.*, 2006]. Amylopectin is a large, branched molecule and forms packed and cluster structure. The cluster structure of the amylopectin is believed to be dependent, among others, on the chain-lengths distribution and branch points [Thompson, 2000; Sanderson *et al.*, 2006]. It was reported that clustering of the branch points appeared in regions of low molecular order. Three types of chains (A, B and C) were found to occur within the cluster structure of amylopectin. Since, the semi-crystalline nature of starch is mainly constituted by amylopectin short branched-chains, this polymer is expected to play a dominating role in starch properties [Srichuwong *et al.*, 2005]. Waxy wheat starch manifested similar amylopectin structure with DP similar to that of non-waxy wheat starch [Bocharnikowa *et al.*, 2003]. However, it was reported that waxy wheat starches required higher energy for gelatinization caused by its higher crystallinity as compared to normal wheat starches and exhibited high resistance to retrogradation [Van Hung *et al.*, 2006]. Singh *et al.* [2006] revealed that waxy maize starch was also characterised by the highest crystallinity compared to maize starches with a high amylose content (normal and sugary maize one). These authors attributed that fact to differences in the proportion of short-, long-side chains of amylopectin and amy-

lose content. According to Singh *et al.* [2006], waxy maize starch manifested higher proportions of long-side chains of amylopectin and short-side amylopectin chains. High crystallinity level of waxy maize starch might have accounted for its lower peak viscosity compared to normal and high amylose ones. The rheological behaviour of high amylopectin potato starch was also found to be significantly different from that of normal potato [Ortega-Ojeda *et al.*, 2004a]. In other work of Ortega-Ojeda *et al.* [2004b], significant differences were shown in rheological behaviour between potato amylose and potato high amylopectin. The strong increment in storage modulus (G') along with the concentration of potato amylose, these authors substantiated by the fact that amylose solution separated first into polymer-rich and polymer-deficient regions, and its chains started to fold and crystallize, triggering the formation of a polymer network. Whereas, potato amylopectin was branched, its external chains were folded and reordered slowly.

It was reported that the properties of starch pastes and gels obtained under high pressure treatment (HP) differed from those of the heat-gelatinized ones [Stolt *et al.*, 1999, 2001]. Błaszczak *et al.* [2005b] showed that the birefringence of waxy maize starch was immediately lost after its treatment at 650 MPa for 3 min in the excess of water. The waxy maize starch subjected to high hydrostatic pressure (650 MPa/3 min) was found to be completely amorphous. Whereas the volume of pressure of 650 MPa was not enough to evoke

a complete destruction of crystallinity of Hylon VII starch [Błaszczak *et al.*, 2005b]. The crystalline structure of potato starch treated with 600 MPa for 3 min was significantly affected by hydrostatic pressure, however the starch retained the granular shape [Błaszczak *et al.*, 2005a]. The differences in behaviour between amylopectin maize starches and amylopectin maize ones can be ascribed to the stabilizing role of amylose macromolecules under high pressure [Stolt *et al.*, 2001]. The pressure-treated starches with medium and high concentration of amylose demonstrated restricted swelling and slight amylose release [Stute *et al.*, 1996]. The high pressure treatment (650 MPa/9 min) of waxy maize starch induced significant disruption of the amorphous and semi-crystalline growth rings, irrespective of their localization within granules, which in turn led to complete gelatinization of granules with the formation of a gel network [Błaszczak *et al.*, 2005a]. Stute *et al.* [1996] noticed that starches of different botanical origin (different type of polymorph) gelatinized at different pressure ranges. Wheat, maize, barley and potato starches treated with high pressure maintained their granular shape and demonstrated limited swelling [Stute *et al.*, 1996; Stolt *et al.*, 2001; Błaszczak *et al.*, 2005a].

It is believed that starches with A-type polymorphous structure are more sensitive to high pressure treatment compared to those of B- and C-type [Stute *et al.*, 1996]. The differences in physico-chemical properties between A- and B-type starches were related to chain length and the branching structure of amylopectin as well as the presence of pinholes and channels on the granule's surface [Jane *et al.*, 2004]. According to those authors, under confocal laser scanning microscopy the waxy maize starch displayed loosely packed structure with large voids around the periphery, whereas the normal maize starch was characterised by more closely packed structure. Jane *et al.* [2004] suggested that the loose structure of waxy maize starch can be attributed to the lack of amylose. It is believed that in "normal" starches the amylose intertwines with amylopectin crystalline clusters and holds amylopectin together to keep the granule's integrity. In the above-cited study the authors paid attention to the fact that the large voids developed in the granule could weaken the structure during physico-chemical treatment.

Far less is known about the effect of high hydrostatic pressure on the structural organization and gelling properties of waxy/amylopectin starches. Bearing in mind that wheat and potato starches are considered as potential and interesting sources for the starch industry [Blennow, 2004; Graybosch, 1998], the specific focus of the work was designed to analyse the structure and gelling properties of amylopectin wheat, potato and waxy maize starch treated with high hydrostatic pressure (650 MPa for 9 min). The physical properties of rehydrated gels obtained from the above-mentioned high pressure-treated starches were investigated as well.

MATERIAL AND METHODS

Material. Amylopectin wheat (APW) and potato (APP) starches with amylose content of 1.5% and 2.1%, respectively, were kindly supplied by Assistant Professor Dr. A. Blennow (Dept. of Plant Biology, Faculty Life Sciences, University of Copenhagen).

The waxy maize (APM) starch (trace amounts of amy-

lose) was purchased from Sigma-Aldrich Sp. z o.o., Poland (catalogue no. S-9679).

Sample preparation. The pressure treatment of starches and starch mixtures (3 g/d.w.) was performed in the excess of water, *i.e.* using a 30% (w/w) starch-water suspension. The suspension was closed into teflon tubs (10 mL), precisely mixed, deaerated, closely sealed and pressure-treated.

Pressure treatment. The pressure treatment of starch-water suspensions was performed in a high pressure device type 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 mL) filled with pressure-transmitting medium which also minimised adiabatic heating. The samples were pressure-treated at 650 MPa for 9 min. The time for reaching the working pressure was 2 min. The temperature inside the pressure chamber averaged $20 \pm 2^\circ\text{C}$. The pressure treatment was performed in two repetitions for each combination.

After the pressure treatment, the starch pastes and/or gels were frozen in liquid nitrogen and freeze-dried.

Structural and thermal characteristics of starch preparations was performed using high sensitivity differential scanning microcalorimetry (HSDSC). The molecular dynamics of water in pressurized gels after their rehydration was studied using $^1\text{H-NMR}$ spectroscopy. The microstructure of pressurized gels was also analysed with scanning electron microscopy (SEM), and its power of swelling was expressed by determination of a swelling index (SWI).

High Sensitivity Differential Scanning Microcalorimetry (HSDSC). The calorimetric investigations of 0.5% aqueous dispersions (sample volume 0.5 mL) of native starches, their mixtures and swelling samples (during 24 h) of starches treated with high pressure were performed with a high sensitive differential scanning microcalorimeter DASM-4 (Puschino, Russia). The measurements were carried out at a temperature range of 10–130°C, excess of a pressure of 2.5 atm., and a heating rate of 2°C/min. Each measurement was performed after calibration of the heat capacity scale. It was shown that under these conditions corrections for dynamic temperature lag and residence time of samples in a calorimetric cell were not necessary [Danilenko *et al.*, 1994; Andreev *et al.*, 1999].

The average values of the thermodynamic parameters (melting temperature and enthalpy) were determined as described elsewhere [Danilenko *et al.*, 1994; Andreev *et al.*, 1999; Matveev *et al.*, 2001], using five measurements at 95% significance level, and converted into per mole of anhydroglucose units (162 g/mol). The baselines for symmetrical and unsymmetrical calorimetric peaks were determined according to the method of Matveev *et al.* [2001].

The values of the gelatinization degree (GD) were calculated using the following equation:

$$\text{GD} = \{(\Delta H_{\text{ns}} - \Delta H_{\text{ts}}) \Delta H_{\text{ns}}^{-1}\} \cdot 100\%$$

where ΔH_{ns} and ΔH_{ts} are the melting enthalpies of native and treated by high pressure starches, respectively.

Determination of a swelling index of samples. The frozen-dried sample (moisture – 8%, weight – 0.020 g) was put into a perforated container made of steel wire, then the container was put into a glass filled with distilled water. At regular intervals, the container was taken out from the glass, whereupon water excess was removed from the surface of the container by filter paper and then the dry container with swelling sample was weighed.

The values of the swelling index (SWI) were calculated using the following equation:

$$\text{SWI} = \{(P_{\text{sws}} - P_0)P_0^{-1}\} \cdot 100\%$$

where P_{sws} and P_0 are the mass of swelling and absolutely dry samples, respectively.

Proton NMR study. Proton relaxation measurements were performed on a low-resolution ^1H -NMR spectrometer (Minispec PC120, Bruker) operating at a proton resonance frequency of 20 MHz. All the measurements were made at $25 \pm 0.1^\circ\text{C}$. The spin-spin relaxation time (T_2) of water protons was measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [Carr & Purcell, 1954; Meibom & Gill, 1958]. PC numerical simulation was used to decompose the experimentally-obtained echo envelope into single exponentials (standard program for Minispec, Bruker). The experimental error in the estimation of T_2 did not exceed 6%.

Scanning Electron Microscopy (SEM). For scanning electron microscopy (SEM), the starch gels/suspension (0.5 mL) were rapidly frozen in liquid nitrogen and freeze-dried. The fractures of dried gels were stuck on a specimen holder using a silver paste, and then coated with gold in a vacuum evaporator (JEE 400, Jeol). The specimens obtained were viewed in a Jeol JSM 5200 scanning electron microscope at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

The DSC thermograms obtained for native APW and APM starch (Figure 1) demonstrated a single and symmetrical peak that was related to the melting of the A-type crystallites [Genkina *et al.*, 2004; Yuryev *et al.*, 2002]. In turn, the peak in the DSC thermogram of native APP starch was assigned to the B-type crystallites since that kind of amylopectin starch shows B-type crystallinity [Bertoft, 2004; Sanderson *et al.*, 2006]. It was postulated that the A-type polymorph with a higher packing density of the double chain helices melted at a higher temperature than the B-type one [Gidley & Bulpin, 1989]. Although, slight differences were demonstrated in melting temperatures between A- and B-type starches which accounted for 7°C [Zobel & Stephen, 1995] or $2\text{--}4^\circ\text{C}$, respectively [Wang & Bogracheva, 1998]. The DSC thermographs obtained for APW and APM starches revealed some differences in melting enthalpy. It is worth mentioning that these starches manifest the same type of crystallinity (A-type). That phenomenon indicated that changes in the crystalline structure of granules were stronger influenced by the botanical origin of starch than by the type of starch polymorph.

Treatment of APM and APW starch with pressure of 650 MPa for 9 min resulted in significant changes in their

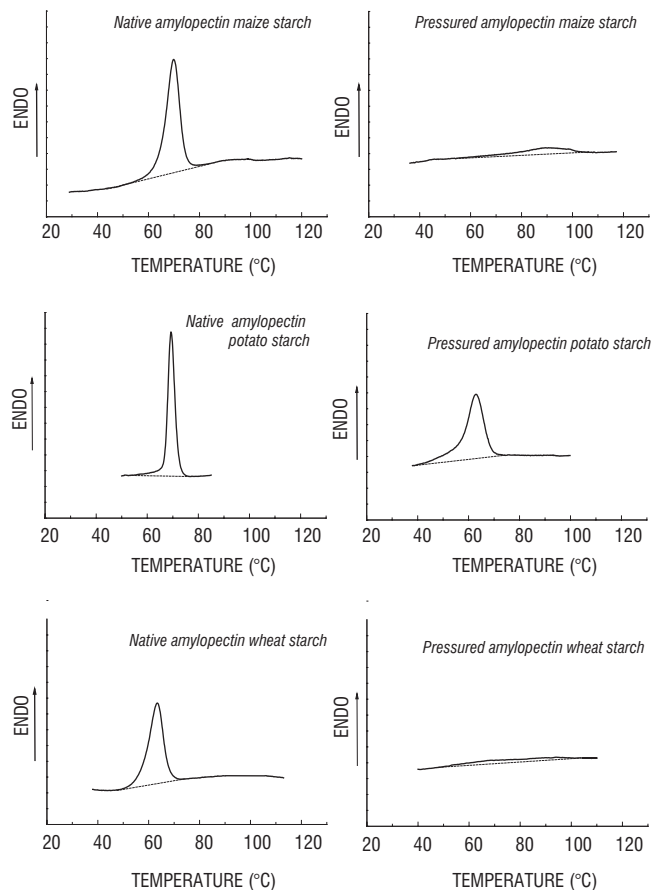


FIGURE 1. DSC-thermographs of native and pressure-treated (650 MPa/9 min) amylopectin starches.

crystalline structure, which in turn affected complete gelatinisation of their granules (Figure 1, Table 1). In contrary, one single peak was still observed in DSC thermogram of pressurised APP starch. However, the peak observed in the DSC thermogram of pressurised APP starch was characterised by lower intensity compared to that appearing in the DSC thermogram of native APP starch. The GD values calculated for pressurised APP starch confirmed the course of DSC thermogram showing that 46% of granules were unaffected by the treatment. The DSC results obtained seemed to be in close relation to the suggestion made by Stute *et al.* [1996]. These authors rated all the B-type starches among the pressure-resistant ones. In contrary, A-type and C-type starches were found to be sensitive to pressure. Differences in susceptibility of A- and B-type starches to high pressure may be assigned to the differences in their granular packing. Indeed,

TABLE 1. The values of the melting enthalpies (ΔH) of native and high pressure-treated amylopectin starches and degree of gelatinisation (GD).

Starch	Polymorphous structure	ΔH (kJ mol $^{-1}$)		GD (%)
		native	pressurized	
Waxy maize	A-type	5.1	0.7	86
AP wheat	A-type	2.8	0.3	90
AP potato	B-type	4.1	1.9	54

Jane *et al.* [2004] has recently indicated that in contrast to B-type starch granules, A-type granules show voids and cracks, reflecting heterogeneous and loose granular packing.

Swelling index determined for the pressurized starches (Figure 2) demonstrated that all the treated amylopectin starches manifested limited swelling properties. Bearing in mind the suggestion of Błaszczak *et al.* [2007], it may be assumed that the limited swelling properties of the analysed starches resulted from a complete breakdown of granules and formation of a gel network, irrespective of their crystalline packing. Limited swelling of such systems (starch-water dispersion) may be ascribed to the presence of the three dimensional gel network that is formed during its heating and subsequent cooling. However, in opposite to that kind of treatment, high pressure-treated amylopectin starches formed the three dimensional gel network already at room temperature. The swelling index of the treated amylopectin starches decreased as follows: amylopectin potato starch \ll amylopectin wheat starch \leq amylopectin maize starch. Taking into account the results of Jane *et al.* [2004], it can be assumed that amylopectin A-type starches (*i.e.* waxy maize and amylopectin wheat starch) with a “loose” granular packing manifested a higher gelling ability compared to the B-type ones (amylopectin potato starch) that presented tightly packed granules.

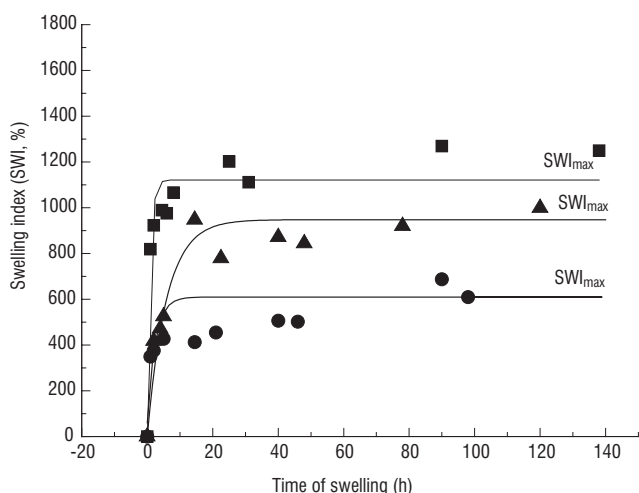


FIGURE 2. Swelling courses of pressure-treated (650 MPa/9 min) amylopectin maize (■), amylopectin wheat (▲) and amylopectin potato (●) starches.

Scanning electron microscopy (SEM) analysis confirmed that high pressure altered the starch granule's structure depending on the type of starch crystallinity (Figure 3). The SEM pictures of waxy maize (APM) and amylopectin wheat (APW) starch after treatment at 650 MPa for 9 min showed a complete breakdown of granules. Pressurization of amylopectin waxy maize and amylopectin wheat starches evoked significant structural changes that resulted in gel formation. That continuous and homogenous gel network was mainly formed by the amylopectin part. In the case of treated amylopectin potato (APP) starch some granules were still observed in microscopy pictures, however, many of them demonstrated significant deformation. Some granules of pressurized APP starch were completely broken and the inner part of granule seemed to be filled with a gel-like structure. Stute

et al. [1996] demonstrated that the treatment of amylopectin maize starch in excess of water at a pressure of 600 MPa led to a complete disintegration of granules. Whereas according to Błaszczak *et al.* [2005], pressurized potato starch (600 MPa/3 min) showed two different zones of its destruction. The outer part of granules (starch surface) was formed by more organized (ordered) structures. According to Svergmak & Hermanson [1991], such surface construction made it more resistant to water-heat treatment. In opposite to surface, the inner part of granules is more complex and consists of growth rings formed by amorphous and crystalline regions. The presence of the amorphous regions in granule structure, hilum (the part of granule most sensitive to hydration) lead to granule hydration and then to the melting of the crystalline structures.

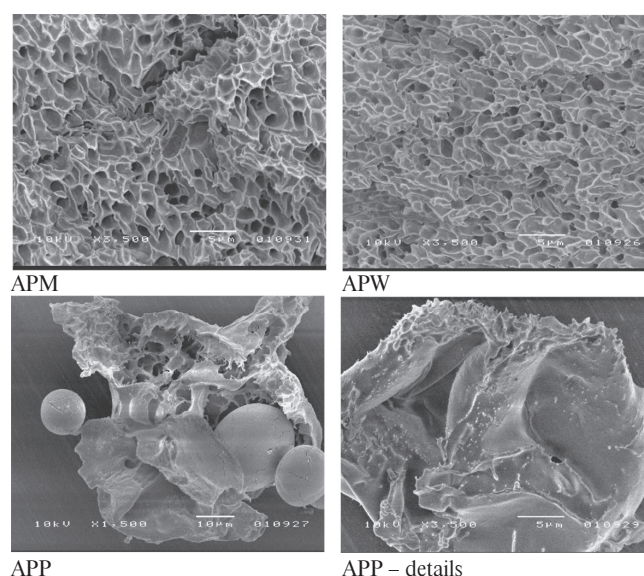


FIGURE 3. Microstructure (SEM) of high pressure-treated (650 MPa/9 min) waxy starches.

The measurements of relaxation time constants (T_2) of the pressurized starches were determined in order to assess the effect of high hydrostatic pressure on water mobility in gel system. The results obtained (Table 2) demonstrated two T_2 values for pressurized and rehydrated starch gels, one with T_2 values between 46 and 720 ms, and the other with values between 8 and 120 ms. The values between 46 and 720 ms were denoted by T_{2a} , whereas the values obtained at shorter relaxation time were assigned T_{2b} . According to Choi & Kerr [2003], spin-spin relaxation is enhanced when, on average, water molecules have hindered rotation and proton spins can exchange energy. Thus, T_{2a} and T_{2b} values were associated with the water molecules differing in their mobility [German *et al.*, 1988, 1992, Choi & Kerr, 2003; Wang *et al.*, 2004]. The T_{2a} values obtained at longer relaxation time can be related to more mobile water, whereas these obtained at the shorter one may be associated with a less mobile water within the analysed gel. According to Choi & Kerr [2003], T_{2a} values were associated with a fairly porous structure – gel network. Whereas the T_{2b} values obtained at shorter relaxation times resulted from a more restricted environment of the granule

TABLE 2. The spin-spin relaxation times of water molecules in pressurized and rehydrated starch gels (650 MPa/9 min).

Starch	T _{2a} (ms)	T _{2b} (ms)
Waxy maize	720	120
AP wheat	431	95
AP potato	46	8

remnant, and/or one that did not rapidly exchange water. It was also demonstrated that the mobility of water molecules in maltodextrin gels (containing no granules) was characterised by two relaxation times [German *et al.*, 1992, 1989]. That kind of water distribution probably resulted from the formation of different structural elements in the pressurised and than in the rehydrated starch gels (*i.e.* aggregates, granule remnants, continuous and porous network) [Błaszczak *et al.*, 2007]. Considering the SWI_{max} and GD values obtained for the pressurized starches, a qualitative correlation may be assumed between these values (Table 1, Figure 2). These data confirmed the suggestion that osmotic properties of the pressurized starches (Table 2) depended, to a significant extent, on the amount of the ordered structures retained after the pressure treatment.

CONCLUSIONS

High pressure-treated waxy maize starch (APM), amylopectin wheat starch (APW) and amylopectin potato (APP) one, manifested different susceptibility to the pressure applied (650 MPa/9 min). The treated APM and APW starches demonstrated almost complete granule gelatinization (GD=86% and 90%, respectively), whereas APP starch treated with high pressure demonstrated GD of 54%. The swelling index of the pressurized starches changed as follows: amylopectin potato starch << amylopectin wheat starch ≤ amylopectin maize starch. The GD and SWI_{max} values showed that the differences in susceptibility to high pressure treatment were significantly related to the type of starch polymorph. The starch gels obtained after pressurization and rehydration may be considered as a complex system consisting of aggregates (granule remnants) and crystalline structures (partially gelatinized and/or intact granules). The differences in gel structure, *i.e.* ratio of granule remnants (swollen structural elements) to the crystalline structures (non-swollen structural elements), significantly affected spin-spin relaxation times of water molecules in the analysed starch gels between the starches.

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WPLYW WYSOKIEGO CIŚNIENIA NA WŁAŚCIWOŚCI I MIKROSTRUKTURĘ ŻELI SKROBI AMYLOPEKTYNOWYCH

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Skrobie: woskową kukurydzianą (APM), amylopektynową pszenną (APW) i amylopektynową ziemniaczaną (APP) poddano działaniu wysokiego ciśnienia (650 MPa/9 min) w nadmiarze wody. Strukturę krystaliczną natywnych i presuryzowanych skrobi, ich zdolności żelowania i właściwości osmotyczne badano przy użyciu wysokoczułej różnicowej mikrokolorimetrii skaningowej (HSDSC) i spektroskopii ¹H-NMR. Skrobie APM i APW poddane działaniu ciśnienia hydrostatycznego, wykazały wysoki stopień skleikowania ziarenek (odpowiednio, GD=86% i GD=90%) i znaczące zmiany w ich strukturze krystalicznej (topnienie krystalitów typu A). Stopień skleikowania ziarenek presuryzowanej skrobi APP wynosił jedynie 54%. Pomiary stałej czasu relaksacji cząsteczek wody w presuryzowanych żelach skrobiowych wykazały dwie różne relaksacje (46–720 ms i 8–120 ms). O złożonych czasach relaksacji cząsteczek wody decydowały różnice w ich mobilności, które bezpośrednio wynikały z różnej struktury badanych żeli, tj. stosunku elementów pęczniących (pozostałości ziarenek) do elementów niepęczniących (struktury krystaliczne). Różnice te wpływały istotnie nie tylko na relaksację cząsteczek wody ale także decydowały o sile pęcznienia rehydratowanych żeli. Stwierdzono, że skrobie amylopektynowe poddane presuryzacji, tworzyły żele, które po rehydratacji wykazały niskie wartości indeksu pęcznienia.