

Modification of granular potato starch by multiple deep-freezing and thawing

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Received 21 January 2002; revised 1 August 2002

Abstract

The influence of multiple freezing of water inside or outside the starch granule on the modification of starch properties has been investigated. Freezing in liquid nitrogen, followed by thawing was repeated in several subsequent cycles for native starch and dried starch–water suspensions (1:1.5 v/v). After such procedures, the precise structural analysis of the starch granule surfaces was carried out by high-resolution non-contact atomic force microscopy. Moreover, gelation characteristics, X-ray diffraction as well as the water and iodine sorption ability of the processed granules were estimated. Pronounced changes were found on the granule surface. After the fifth freezing, a well-ordered microstructure of the surface with distinct straight chains of fine particles of about 30 nm in diameter could be observed. The surface regularities were accompanied by some irreversible disruption of the granule inner structure indicated by changes of the starch crystallinity and in water adsorption. The freezing/thawing process also influenced the gelation characteristics, water solubility and water holding capacity, but did not change the branching characteristics of the studied starch granules.

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Keywords: Non contact-atomic force microscopy; Potato starch; Potato starch granule surface; Starch granule modification

1. Introduction

Freezing is a physical treatment widely applied for preservation, drying and lyophilisation of starchy food (Ahmed & Lelievre, 1978; Biliaderis, Swan, & Arvanitoyannis, 1999). It is also used for sample preparation in granule structural investigations by means of many physical methods, for instance in scanning electron microscopy (SEM) or transmission electron microscopy (TEM) (Conde-Petit, Nuessli, Handschin, & Escher, 1998; Gallant & Guilbot, 1971; Gallant, Bouchet, & Baldwin, 1997; Oostergetel & van Bruggen, 1993). It was reported that freezing influenced textural and gelatinisation characteristics of starch (Jericevic & Oraikul, 1977; Tomasik & Zaranyika, 1995). It was also considered to cause some changes in the nutritional properties of starch (Guraya, James, & Champagne,

2001). Freezing of starch sols resulted in their coacervation and increasing retrogradation, while pregelatinised starch became less sensitive to retrogradation and stayed smooth after the process (Tomasik & Zaranyika, 1995). Waigh, Perry, Riekkel, Gidley, and Donald (1998) as well as Perry and Donald (2001) reported that some reversible structural disorder of starch granules occurred at sub-zero temperatures. Freezing water inside or outside of the starch granule seems to be a particularly effective way for modification for potato starch. This is because the starch B granules contain a significant amount of structural water, which determines the granular inner structure. Recently, we have observed that deep-freezing of granular potato starch significantly influences the granule surface (Krok, Szymońska, Tomasik, & Szymoński, 2000; Szymońska, Krok, & Tomasik, 2000). Results strongly depended on water content in the sample. We assumed that the freezing/thawing procedure could be more effective when repeated for moist granular potato starch in several subsequent cycles. Thus, samples

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of native starch (12% w/w moisture content) and oven-dried starch (8% w/w moisture content) –water suspensions (1:1.5 v/v) were frozen with liquid nitrogen, thawed in the open to room temperature, and dried over KOH. After that small and wide angle X-ray diffraction, pasting characterisation, water and iodine sorption, and intrinsic viscosity of the samples were determined. Moreover, a precise structural analysis of the starch granule surface was carried out by high-resolution non-contact atomic force microscopy (nc-AFM). The AFM technique can be used for direct observation of the starch granule surface details without any destructive preparation of the sample (Baldwin, Adler, Davies, & Melia, 1998; Gallant et al., 1997; Morris, Kirby, & Gunning, 1999; Ohtani, Yoshino, Hagiwara, & Maekawa, 2000). Use of nc-AFM imaging method allows for the minimisation of the interaction of the sample surface with the AFM tip (Krok et al., 2000).

2. Experimental

2.1. Materials

Commercially available potato starch was isolated in Nowamyl (Poland) according to Polish Standard PN-A-74710. It was used either in the air-dried state (native starch 12% w/w of moisture) or after drying in the oven at 130 °C for 3 h (8% w/w of moisture).

2.2. Freezing/thawing procedure

Potato starch samples were frozen according to a formerly described procedure (Szymoniska et al., 2000). Oven-dried potato starch (~5 g) was quickly blended with water (water/starch 1.5:1 v/v) and liquid nitrogen poured on to it in an open container. The native (air-dried) starch samples were frozen only with their original water. After nitrogen evaporation, the samples were allowed to equilibrate for about 0.5 h to room temperature. Then the freezing/thawing procedure was repeated no more than five times for starch–water suspensions, and twice for native starch. Samples were dried with filter paper as well as over KOH, and stored in closed vessels for further investigations.

2.3. Non-contact atomic force microscopy

High-resolution nc-AFM was performed using a Park Scientific Instrument Autoprobe CP model (California, USA) of the Regional Laboratory for Physicochemical Analyses and Structural Research at the Jagiellonian University, as described elsewhere (Krok et al., 2000; Szymoniska et al., 2000). Potato starch granules were spread onto adhesive tape fixed onto an AFM sample holder, and observed at ambient conditions. The granules were partially

embedded in the ‘sticky tape’ to overcome problems with the large height variation in granule topography. For each starch sample images of several starch granules were collected.

2.4. X-ray diffraction patterns

X-ray diffraction patterns for starch powders were recorded at room temperature with a Phillips diffractometer X’pert-type using the Cu K α line at $\lambda = 1.5418 \text{ \AA}$ (20 mA and 50 kV). The scanning region of 2θ from 3 to 57° in 0.04° intervals and counting time of 2 s/step was used for wide angle grazing incidence diffraction (GID). For small angle diffraction measurements 2θ from 0.25 to 7.75 ° in 0.005° intervals and counting time of 5 s/step was applied. The measurements were performed at the laboratory of the Electronics Department of the University of Mining and Metallurgy (AGH) in Krakow, Poland.

2.5. Moisture sorption isotherms

Moisture sorption isotherms were taken at 298 K by using a volumetric type apparatus with adsorption micro-burettes as described by Ciembroniewicz and Komorowska-Czepirska (1985). Prior to experimental study samples were degassed at 343 K to the residual pressure of about 10^{-4} mm Hg.

2.6. Characteristics of gelation

Gelation was characterised for 3.2% aqueous starch suspensions using a Rheotest 2 rotary viscometer (Pruefger-aete-Werk Medingen, Sitz-Freital, Germany) (Szymoniska et al., 2000). The standard program was applied with temperature increasing from 50 to 96 °C at a rate of 1.5 °C/min, followed by a 20 min relaxation at 96 °C and then cooling with the same rate to 50 °C. Gel viscosity was also measured after 10 min storage at this temperature.

2.7. Intrinsic viscosity

Intrinsic viscosity was determined at 25 °C (± 0.1 °C) according to method described by Carriere (1998), by using a modified Zimm rotary viscometer. Solutions for the measurements were prepared from 100 mg of starch, which was added to 10 cm³ of distilled water in a volumetric flask. With gentle agitation 60 cm³ of dimethylsulfoxide (DMSO) was added. The flask was gradually heated to 80 °C over a period of 20–30 min and held for 3 h in this temperature. After cooling to 25 °C, an additional amount of DMSO was added to bring the total final volume of the solution to 100 cm³. This stock solution was diluted with DMSO–water (90:10 v/v) solvent to the desired concentrations. For each sample seven different solutions in the concentration range from 0.0 to 0.1 g/dm³ were investigated. Intrinsic viscosity was obtained by plotting the reduced viscosity

Dried Potato Starch

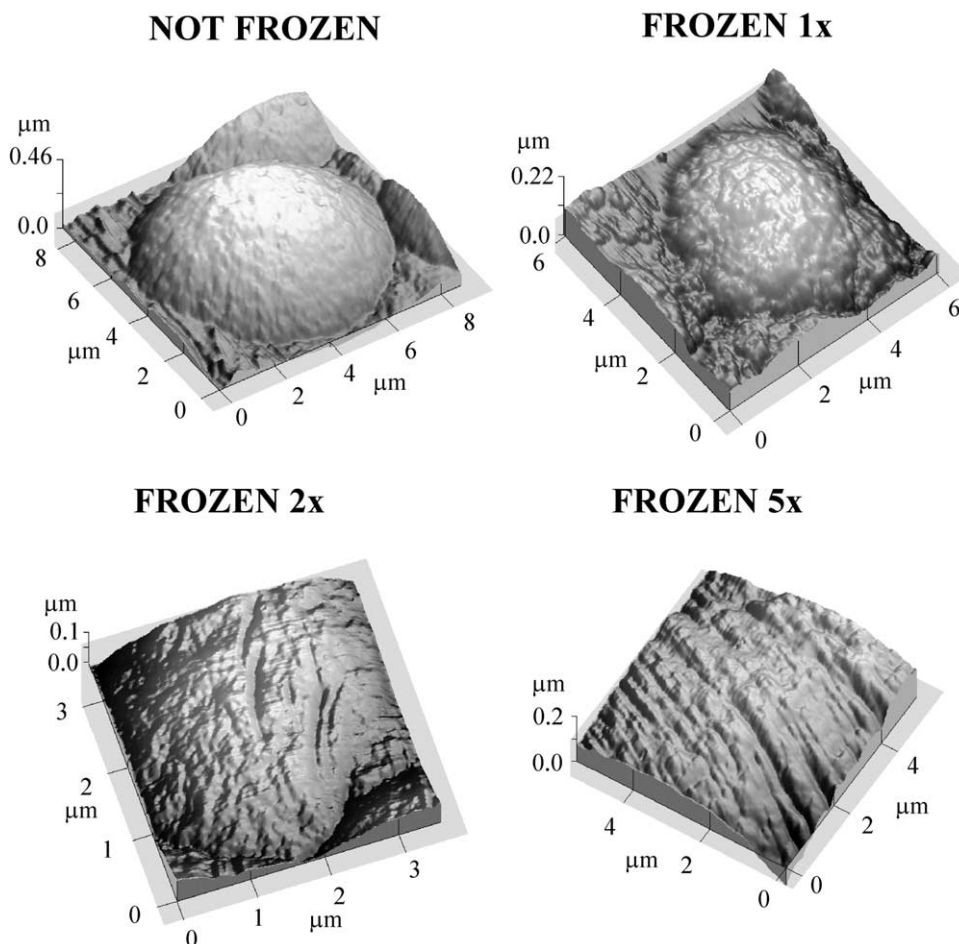


Fig. 1. nc-AFM images of the dried potato starch granule surface changes after multiple freezing/thawing in aqueous suspension.

versus concentration and extrapolation to zero concentration by a linear regression.

2.8. Water solubility and water holding capacity

Water solubility and water holding capacity of the samples were measured following the method described by Richter, Augustat, and Schierbaum (1968).

2.9. Iodine staining

Branching characteristics of starch was determined by iodine complexing combined with spectroscopic quantification of the absorption at 525 nm (E_{525}) and 640 nm (E_{640}) according to method described by Praznik, Mudigler, Kogler, Pelzl, and Huber (1999). Absorption measurements were performed using a Shimadzu 2101 PC UV–Vis spectrophotometer.

The ‘blue value’ was obtained according to Klucinec and Thompson (1998). Starch samples (40 mg) were dispersed

in 10 cm³ of DMSO containing 10% 6 M urea. A 1.0 cm³ aliquot of each sample was placed in a 100 cm³ volumetric flask, to which 95 cm³ of de-ionised water and 2 cm³ of an aqueous I₂–KI solution were added. The latter solution contained of I₂ (200 mg) and KI (2 g) in distilled water (100 cm³). The mixture was brought to 100 cm³ with de-ionised water and mixed immediately. Absorption spectra of the samples were taken in the wavelength range of 500–800 nm with Shimadzu 2101 PC UV–Vis spectrophotometer. The blue value was defined as the sample absorbance at 640 nm. Blank solutions not containing starch were prepared identically. The measurements were run threefold.

3. Results and discussion

The most pronounced changes after several freezing/thawing cycles appeared on the granule surface (Figs. 1 and 2). The surface looked smooth and polished

Dried Potato Starch

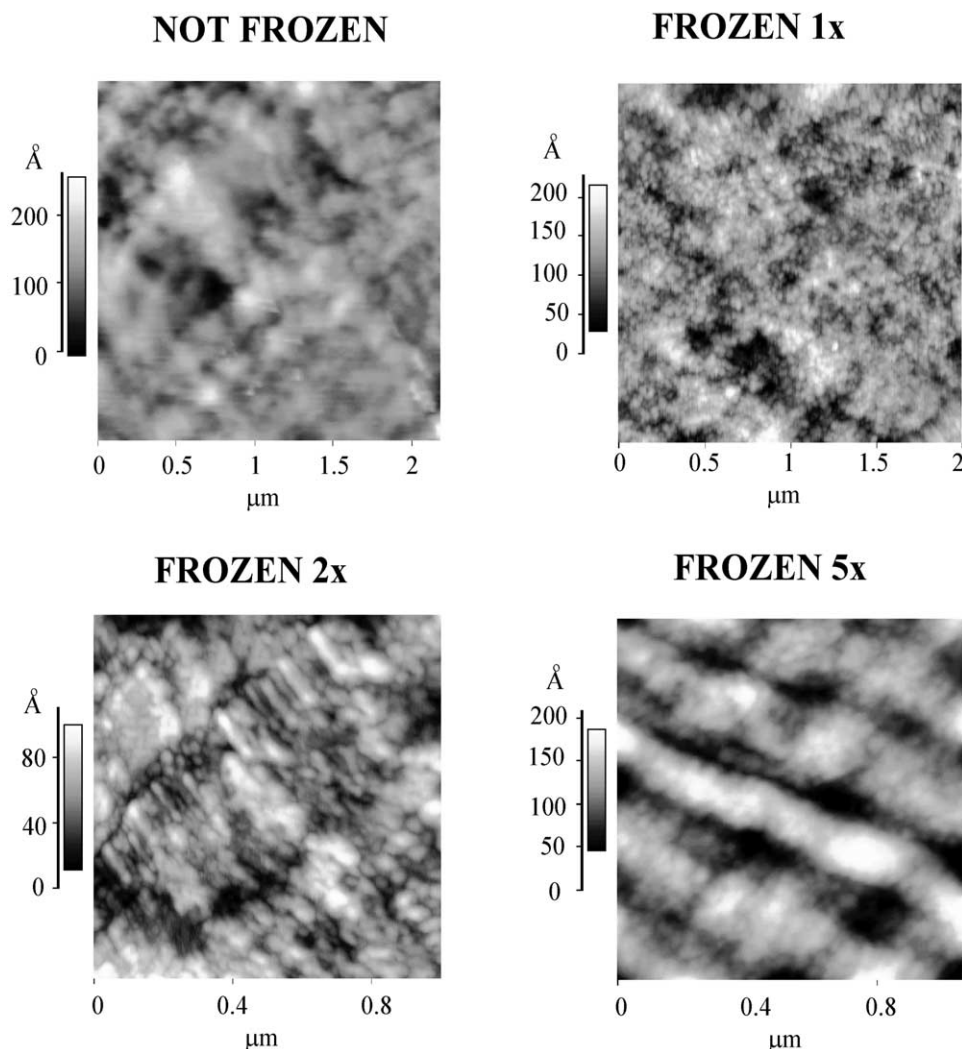


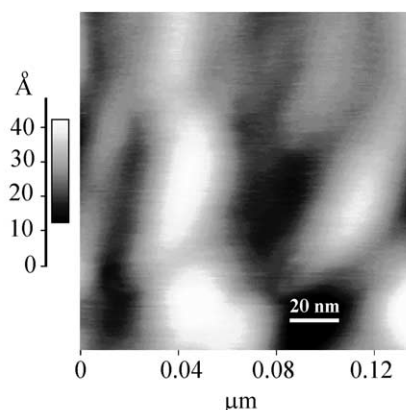
Fig. 2. nc-AFM images of the dried potato starch granule surface structure after multiple freezing/thawing in aqueous suspension.

after the first freezing step and consisted of randomly distributed sub-particles (Fig. 2). The subsequent freezing/thawing cycle caused more roughness and oblong scratches developed on the surface. Finally, after the fifth freezing, a well-ordered structure of the surface with the distinct straight chains of fine particles was revealed (Figs. 2 and 3). Similar prolonged nodules of about 30 nm in diameter could be observed for all frozen starch samples (Figs. 3 and 4). The structure of these surface elements is not yet known. However, due to their dimensions, they may be attributed to single carbohydrate helical clusters or blocklets containing several amylopectin side chain clusters. These findings are in accordance with the formerly presented results and proposed models of starch granule structure (Baldwin et al., 1998; Gallant et al., 1997; Ohtani et al., 2000; Oostergetel & van Bruggen, 1993).

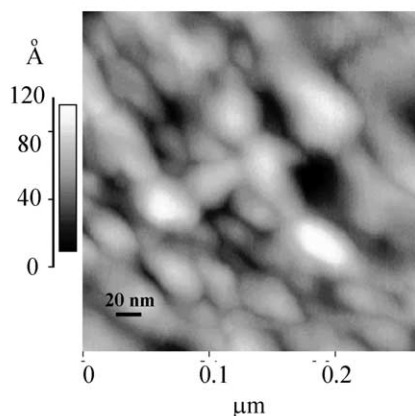
The surface scratches and roughness were most likely caused by expansion of the granule under the influence of ice formed from water occupying their amorphous lamellae or wall channels. For starch aqueous suspensions, such an effect was accompanied by compression of the granule by the matrix of freezing external water. This might cause disruption of the crystalline arrangement inside the starch granule (long-range order structures) and weakening of interactions between double helices (short-range order structures) on thawing as it was suggested by Bogracheva, Wang, and Hedley (2001). The result became more pronounced after each freezing/thawing cycle. This is just opposite to the findings reported by Donald, Kato, Perry, and Waigh (2001) and Perry and Donald (2001) who implied that the loss of the granule structure organisation on freezing could be reversed upon reheating. Granules disordered on freezing/thawing should be readily

Dried Potato Starch

FROZEN 1x



FROZEN 2x



FROZEN 5x

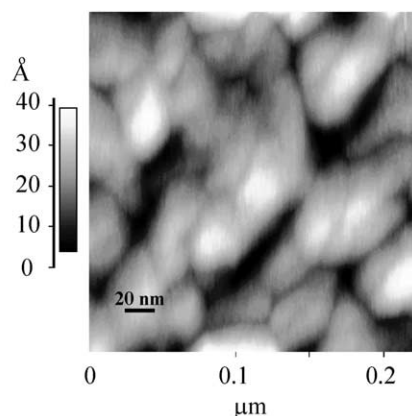


Fig. 3. nc-AFM images of the fine particles of about 30 nm at the dried potato starch granule surface after multiple freezing/thawing in aqueous suspension.

accessible for water molecules present in the system, i.e. external as well as those released from the amorphous part of the granule, because new paths for their penetration into granule interior were opened. Under the experimental conditions used, the water molecules might form new intra- and/or inter-molecular hydrogen bonds protecting structural arrangements in the granule. This could result in a nematic–smectic phase inter-conversion of double helical clusters and better macro-organisation of the sample, which is manifested by the granule surface regularities as well as by the higher starch crystallinity. Fig. 5 presents X-ray diffraction patterns obtained for the samples under study. Dried starch ($\sim 8\%$ w/w moisture content) frozen without any additional water did not change its X-ray diffraction patterns (Fig. 5a–line 2). On the contrary, a remarkable increase in crystallinity could be seen for the dried starch frozen in aqueous suspension (Fig. 5a–lines 3 and 4). In that case, the amorphous area of the diffractograms was significantly reduced and a peak

at 2θ of 5.5° (for 1.58 nm) could be seen (Fig. 5b). This peak corresponds to the (100) crystallographic planes and is typical for moisturised starches (Cleven, van der Berg, & van der Plas, 1978; Nara, 1978). The relative height of the peak increased with the number of freezing/thawing cycles. It could be observed even though the sample moisture content, increased rapidly after first freezing, was constant at a similar level after the next cycles (Table 1). Our observations lead to the conclusion that freezing of starch in aqueous suspension is causing water redistribution in the starch granule due to incorporation of water molecules into the newly formed semicrystalline structures. This is in agreement with our previous results obtained by NMR relaxation for potato starch after freezing/thawing (Banaś, Blicharska, & Szymońska, 2001).

Water adsorption isotherms taken for all the investigated samples exhibited a type II character (according to IUPAC classification) (Fig. 6a) and fit very well the BET

Native Potato Starch

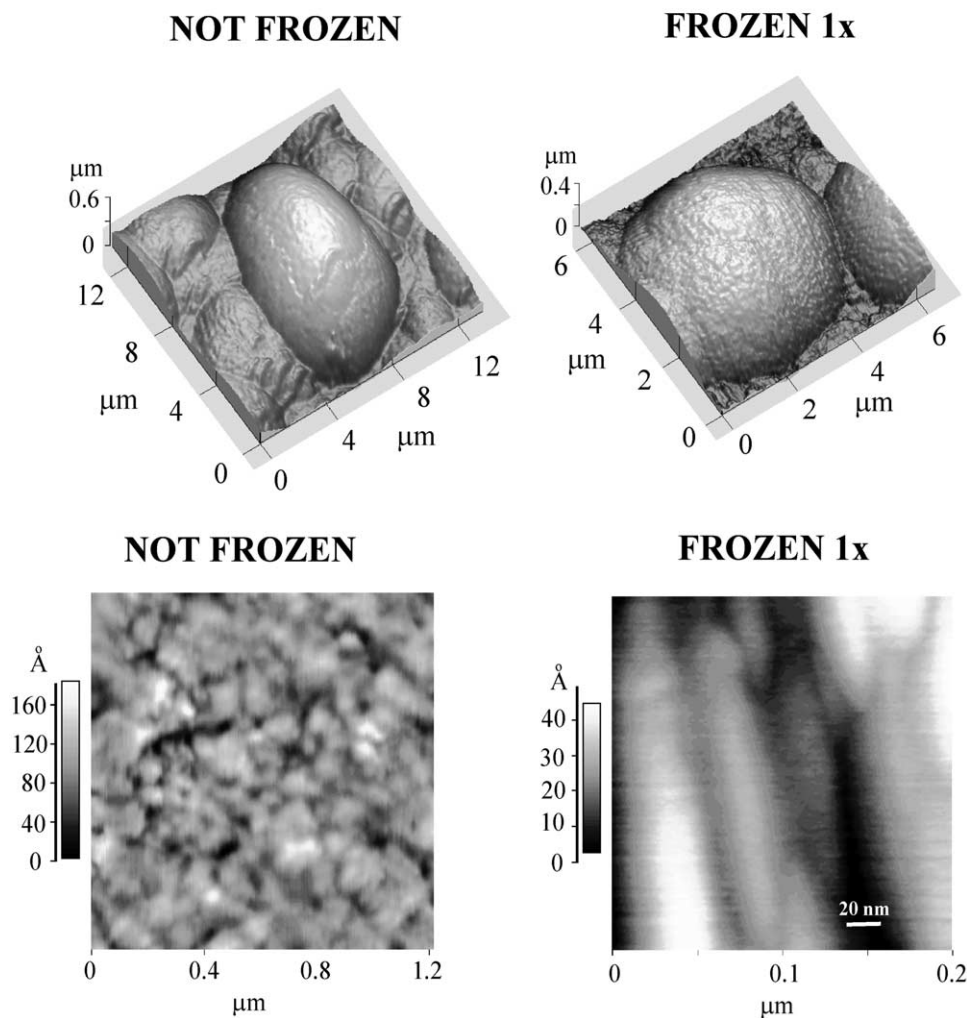


Fig. 4. nc-AFM images of the native potato starch granule surface structure after freezing/thawing.

theoretical equation. The isotherms resulted from an unrestricted monolayer–multilayer adsorption at hydroxyl groups of the glucose residues on the amorphous part and the surface of the crystallites in the starch granule (Nara, 1978). A visible reduction of evaluated monolayer adsorption at primary sites (a_p), with simultaneous increasing of adsorption at secondary sites (a_s), occurred for native starch as well as for starch–water suspensions just after first freezing (Fig. 6(b) and (c)). It was found to be independent of the number of freezing/thawing cycles. This could be caused, not only by the reduction of the granule amorphous part in the process, but also by a lower number of effective hydroxyl groups which might be involved in new hydrogen bond formation, and thus became unavailable as active primary adsorption sites.

Gelation characteristics of 3.2% aqueous starch suspensions are presented in Table 2. Gels formed from

frozen native starch were more viscous and less sensitive to retrogradation than those formed from dried starch frozen in aqueous suspension. In each case, however, the most significant differences were obtained after the first freezing. Lower viscosity observed for gels formed from starch frozen in aqueous suspension could be caused by higher disintegration of the granules in this process compared to the native starch granules. This effect disappeared on subsequent cycles because water molecules present in the system took part in the formation of a new inter-granular network of hydrogen bonds stabilising the granules. In native starch, the hydrogen bonds were formed with a share of much lower amount of structural water molecules released from the granule amorphous region during the freeze/thawing process.

Only minor changes in intrinsic viscosity and blue value (this is, absorption of starch–iodine complexes at

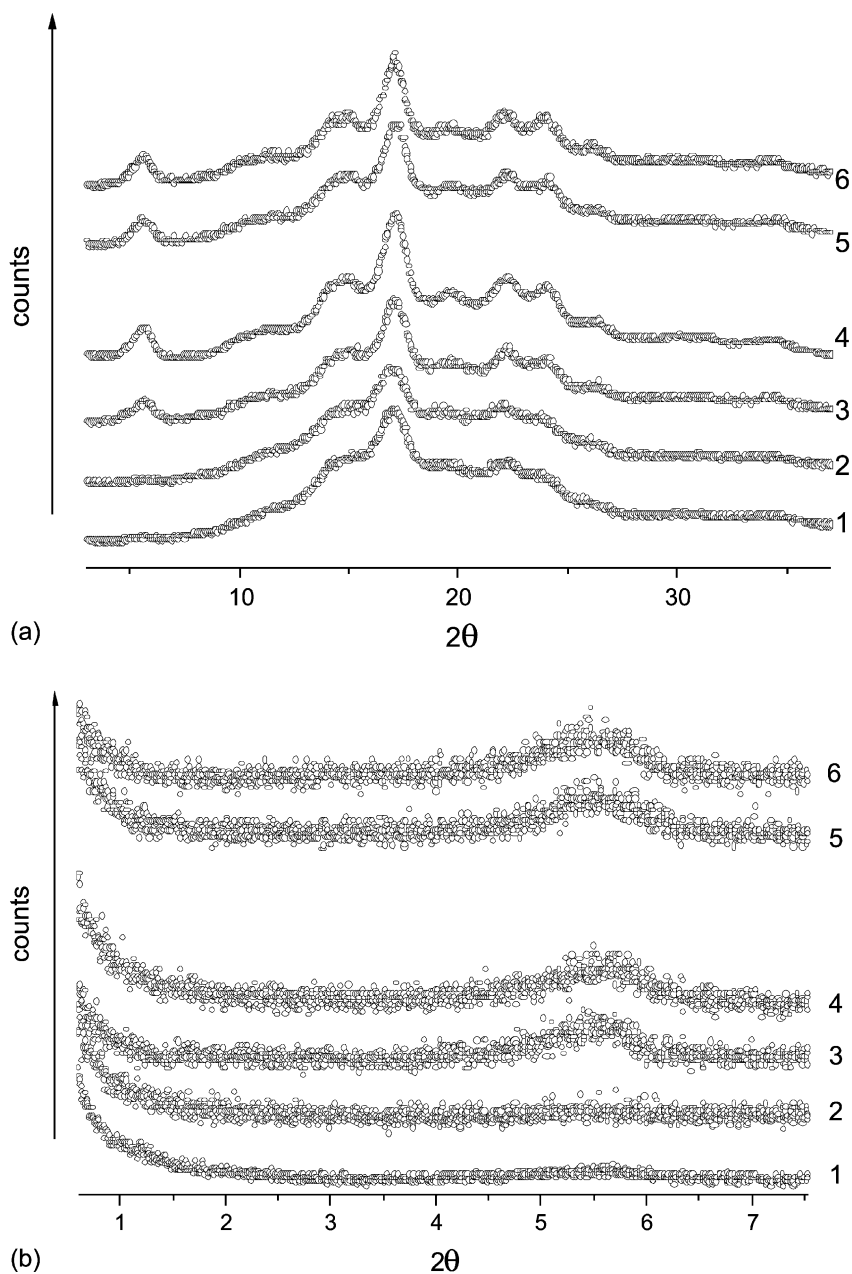


Fig. 5. Wide angle (5a) and small angle (5b) X-ray diffraction patterns of investigated potato starch samples. (1) Oven-dried not frozen; (2) oven-dried frozen without water; (3) oven-dried frozen 1 × in aqueous suspension; (4) oven-dried frozen 5 × in aqueous suspension; (5) native—not frozen and (6) native—frozen 1 ×.

$\lambda = 635$ nm) for starch after freezing/thawing could be noted (Table 3). For all samples, the iodine complexing potential of high molecular components, expressed as the E_{640}/E_{525} ratio (correlated branching characteristics) (Praznik et al., 1999), has not exceeded the value of 1.5. This indicates that the amylopectin-type short-chain branched glucans has not been significantly influenced by the freeze-thaw procedures.

Water solubility and water holding capacity estimated for the samples, were slightly higher after the first freezing step and then decreased after subsequent cycles.

Table 1
Sample moisture content and the relative height of the 1.58 nm peak after freezing/thawing procedures

Potato starch sample	Sample moisture content (% w/w)	Relative height of 1.58 nm peak ± 0.05
Dried (not frozen)	7.89	0.36
Starch–water suspension (frozen 1 ×)	12.10	1.25
Starch–water suspension (frozen 5 ×)	13.15	1.40
Native starch (not frozen)	12.14	1.21
Native starch (frozen 2 ×)	11.87	1.29

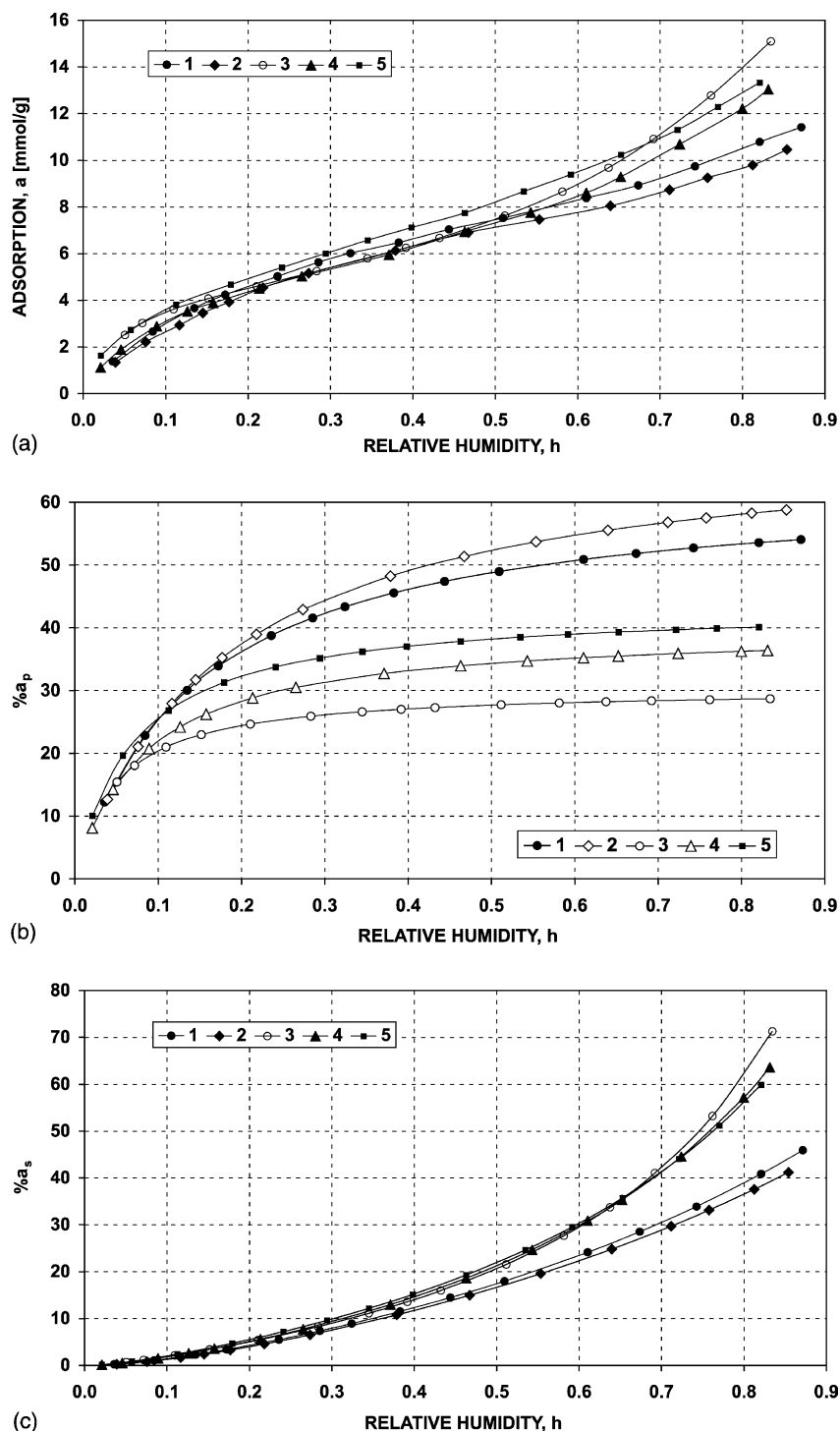


Fig. 6. Water sorption isotherms obtained for the investigated starch samples. (a) (1) oven-dried not frozen; (2) native—not frozen; (3) dried—frozen $1 \times$ in aqueous suspension; (4) native—frozen; (5) oven-dried—frozen without water. (b) Evaluated water adsorption at primary sites (a_p) for the investigated starch samples. (c) Evaluated water adsorption at secondary sites (a_s) for the investigated starch samples. (1) Oven-dried not frozen; (2) native—not frozen; (3) dried—frozen $1 \times$ in aqueous suspension; (4) native—frozen and (5) oven-dried—frozen without water.

4. Summary

1. The multiple freezing/thawing process significantly modified the granule surface as well as causing weakening of double helices, and produced irreversible

disruption of the crystalline order within the potato starch granule.

2. The process altered the water distribution in the granule due to the incorporation of released water molecules into the newly formed semicrystalline structures. Formation

Table 2
Gelation characteristics of 3.2% aqueous starch suspensions on Rheotest 2 rotary viscometer

		Potato starch variety						
		Dried not frozen	Dried frozen in water suspension			Native		
			1 ×	3 ×	5 ×	Not frozen	Frozen	
							1 ×	2 ×
Gelation temperature (°C)		62.5	63.5	63.0	63.0	65.0	63.0	63.0
Viscosity (a.u.) ^a	Maximum	45.5	35.9	42.6	43.0	42.9	54.2	53.0
	Initial at 96 °C (η_{96})	42.7	32.3	38.2	39.0	35.1	58.3	52.4
	After 20 min at 96 °C	31.1	29.9	33.4	32.4	36.1	32.6	33.0
	Initial at 50 °C (η_{50})	37.3	35.0	40.2	39.0	44.5	39.0	39.4
	After 10 min at 50 °C	37.7	36.2	41.0	39.8	45.7	38.6	40.6
η_{50}/η_{96}		0.87	1.08	1.05	1.00	1.27	0.72	0.75
Increase in $\eta_{96} \rightarrow \eta_{50}$ (%)		21.2	21.0	22.7	22.8	26.6	18.4	23.0

^a Arbitrary units (a.u.).

Table 3
Molecular characteristics of investigated starch–water suspensions

	Potato starch variety						
	Dried not frozen	Dried frozen in water suspension			Native		
		1 ×	3 ×	5 ×	Not frozen	Frozen	
						1 ×	2 ×
Intrinsic viscosity at 25 °C (cPcm ³ /mg)	2.3	1.8	1.9	1.8	2.0	2.0	2.0
‘Blue value’ (E_{635})	0.32	0.31	0.28	0.29	0.28	0.30	0.31
Ratio E_{640}/E_{525}	1.50	1.47	1.51	1.54	1.56	1.53	1.51

of new hydrogen bonds utilised effective hydroxyl groups of glucose units in the granule and influenced the sorption ability of the investigated potato starch. This might influence the granule surface through processes such as protein or salts binding to the starch.

3. Repeated freeze–thawing procedures slightly influenced the water solubility and the water holding capacity, but did not change the branching characteristics of starch granules.
4. Gels formed from frozen native starch were more viscous and less sensitive to retrogradation than those formed from dried starch frozen in aqueous suspension.
5. Except for the surface structure, the most pronounced modification of granular potato starch properties was obtained after first the freezing/thawing process.

Acknowledgements

This work is a part of the project No. 5 P06G 07319 supported by the Polish State Committee for Scientific Research. We would like to express our gratitude to Prof. P. Tomasik who shared his ideas with us, motivated the investigation and discussed the results.

Prof. T. Stobiecki is gratefully acknowledged for making possible the powder X-ray diffraction measurements performed at the laboratory of the Electronics Department of the University of Mining and Metallurgy (AGH) in Krakow, Poland.

References

- Ahmed, M., & Lelievre, J. (1978). Effect of various drying procedures on the crystallinity of starch isolated from wheat grains. *Starch/Stärke*, 30(3), 78–79.
- Baldwin, P. M., Adler, J., Davies, M. C., & Melia, C. D. (1998). High resolution imaging of starch granule surfaces by atomic force microscopy. *Journal of Cereal Science*, 27, 255–265.
- Banas, K., Blicharska, B., & Szymońska, J. (2001). NMR relaxation of potato starch samples after freezing. *Molecular Physics Reports*, 33, 165–168.
- Biliaderis, C., Swan, R., & Arvanitoyannis, I. (1999). Physicochemical properties of commercial starch hydrolytes in the frozen state. *Food Chemistry*, 64, 537–546.
- Bogacheva, T. Y., Wang, Y. L., & Hedley, C. L. (2001). The effect of water content on the ordered/disordered structures in starches. *Biopolymers*, 58, 247–259.
- Carriere, C. J. (1998). Evaluation of the entanglement molecular weights of maize starches from solution rheological measurements. *Cereal Chemistry*, 75(3), 360–364.

- Ciembroniewicz, A., & Komorowska-Czepirska, E. (1985). Aparatura do badania równowag i kinetyki sorpcji w szerokim przedziale temperatur. *Przemysł Chemiczny*, 6, 62–65.
- Cleven, R., van der Berg, C., & van der Plas, L. (1978). Crystal structure of hydrated potato starch. *Starch/Stärke*, 30(7), 223–228.
- Conde-Petit, B., Nuessli, S., Handschin, S., & Escher, F. (1998). Comparative characterisation of aqueous starch dispersions by light microscopy, rheometry and iodine binding behaviour. *Starch/Stärke*, 50(5), 184–192.
- Donald, A. M., Kato, K. L., Perry, P. A., & Waigh, T. A. (2001). Scattering studies of the internal structure of starch granules. *Starch/Stärke*, 53(10), 504–512.
- Gallant, D. J., & Guilbot, A. (1971). Artefacts au cours de la preparation de coupes de grain d'amidon. Etude par microscopie photonique et electronique. *Starch/Stärke*, 23, 244–250.
- Gallant, D. J., Bouchet, B., & Baldwin, P. M. (1997). Microscopy of starch: Evidence of a new level of granule organization. *Carbohydrate Polymers*, 32, 177–191.
- Guraya, H. S., James, Ch., & Champagne, E. T. (2001). Effect of cooling, and freezing on the digestibility of debranched rice starch and physical properties of the resulting material. *Starch/Stärke*, 53, 64–74.
- Jericevic, D., & Oraikul, B. (1977). Influence of the processing on the surface structure of potato granules as viewed by SEM. *Die Stärke*, 29(5), 166–172.
- Klucinec, J., & Thompson, D. B. (1998). Fractionation of high amylose maize starches by differential alcohol precipitation and chromatography of the fractions. *Cereal Chemistry*, 75(6), 887–896.
- Krok, F., Szymońska, J., Tomasik, P., & Szymoński, M. (2000). Non-contact AFM investigation of influence of freezing process on the surface structure of potato starch granule. *Applied Surface Science*, 157, 382–386.
- Morris, V. J., Kirby, A. R., & Gunning, A. P. (1999). *Atomic force microscopy for biologists*. Imperial College Press, chapter 4; pp. 121–123.
- Nara, S. (1978). On the relationship between regain and crystallinity of starch. *Starch/Stärke*, 30(6), 183–186.
- Ohtani, T., Yoshino, T., Hagiwara, S., & Maekawa, T. (2000). High-resolution imaging of starch granule structure using atomic force microscopy. *Starch/Stärke*, 52(5), 150–153.
- Oostergetel, G. T., & van Bruggen, E. F. J. (1993). The crystalline domains in potato starch granules are arranged in a helical fashion. *Carbohydrate Polymers*, 21, 7–12.
- Perry, P. A., & Donald, A. M. (2001). The effect of low temperatures on starch granule structure. *Polymer*, 41, 6361–6373.
- Praznik, W., Mudigler, N., Kogler, A., Pelzl, B., & Huber, A. (1999). Molecular background of technological properties of selected starches. *Starch/Stärke*, 51(6), 197–211.
- Richter, M., Augustat, S., & Schierbaum, F. (1968). *Ausgewählte Methoden der Stärkechemie*. Leipzig: Veb Fachbuchverlag, pp. 47.
- Szymońska, J., Krok, F., & Tomasik, P. (2000). Deep freezing of potato starch. *International Journal of Biological Macromolecules*, 27, 307–314.
- Tomasik, P., & Zaranyika, M. F. (1995). Nonconventional methods of modification of starch. In D. Horton (Ed.), (51) (pp. 296–298). *Advances in carbohydrate chemistry and biochemistry*, New York: Academic Press.
- Waigh, T. A., Perry, P. A., Riekel, Ch., Gidley, M. J., & Donald, A. M. (1998). Chiral side-chain liquid-crystalline polymeric properties of starch. *Macromolecules—Communications to the Editor*, 31, 7980–7984.