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Changes in the microstructure of wheat, corn and potato starch granules during extraction of non-starch compounds with sodium dodecyl sulfate and mercaptoethanol

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Abstract

Wheat, corn and potato starches were subjected to multiple extractions with 1% sodium dodecyl sulfate and mercaptoethanol (SDS/ME) in order to remove non-starch compounds from the starch granule. Molecular mass distribution of the extracted material determined using the column chromatography (Sepharose CL-2B) showed two distinct peaks of 200–800 kDa for high- (HMW) and 100–120 kDa for low molecular weight material (LMW). Only in wheat starch extract (onefold extraction) analysed using ^{1}H- and ^{13}C NMR spectroscopy, in phospholipid area, the main component of LMW was lysophosphatidylcholine (LPC). The residual starch pellets after fifteenfold extraction with SDS/ME were examined by the column chromatography, X-ray diffraction, and light microscopy. Amylopectin insoluble in solvent used was the main component of starch granules residue after extraction. The extraction of non-starch compounds did not affect changes in A-type and B-type crystallinity of cereal and tuber starches, respectively. The starch pellets were characterised by higher relative crystallinity compared to native ones. The removal of non-starch compounds resulted in different behaviour of starch pastes during heating at gelatinisation temperatures.

A solvent mixture of *n*-propanol: water (3:1, v/v) was also used in order to extract surface lipids (cold extraction) as well as lipids present in the starch granule interior (hot extraction). The highest amount (58.8%) of surface lipids was found in wheat starch, while the lowest—in potato starch (9.9%).

Keywords: Starch granule; Amylose; Amylopectin; Surface compounds; Structural study

1. Introduction

It is well known that starch granules are semicrystalline particles in which glucose is polymerized into amylose and amylopectin, forming closely packed and ordered structure of granule (Bogracheva, Wang, & Hedley, 2001; Gallant, Bouchet, Buleon, & Perez, 1992; Hermansson, Kidman, & Svegmark, 1995). Nowadays more and more attention is paid to the role of carbohydrate and non-starch compounds in maintaining the 3-dimensional granule form (Seguchi & Kanenaga, 1997). Atkin, Cheng, Abeyssekera, and Robards (1999) reported that in the starch structure there could be distinguished three structural regions: crystalline region (double helical), amorphous (branched and amylose regions), and solid one (consisting of lipid inclusion complex). It has been suggested that a unique structure of starch granules, different for each botanical source, strongly influences their chemical and enzymatic reactivity. Svegmark (1992) reported that starch contains also intermediate types of molecules that cannot be defined as amylose or amylopectin. In maize starch, for example, 5–7% of the polysaccharides are of the intermediate character, and also this type of material was found in potato as well as in wheat starch. Seguchi (1995) and Seguchi and Kanenaga (1997, 1998) used starch-Remazolbrilliant Blue R-dye (RBB) complex, and next its stepwise extraction with sodium dodecyl sulfate and mercaptoethanol (SDS/ME) for starch

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granule characterization. RBB is well known as reagent binding the polysaccharide covalently (hydroxyl group of starch and hydroxyethyl sulfonyl group of RBB) and thanks to that also this as a good marker for surface molecules. Mixture of SDS/ME was chosen in order to dissolve and remove the trace materials located on the surface of starch granules that are crucial for maintaining their structural stability. After removal of the surface compounds the loosening of the granule interior is expected so deeper penetration of the specific solvent (SDS/ME) take place and extracts could be characterized for their profiles. Mentioned above authors showed that the starch granule interior consists mainly of high-molecular-weight (HMW) carbohydrate material. This material is arranged within the granules what can be visualized by removing soluble surface compounds with the solvent used and spliting the granules into two distinct halves. It was shown that the surface of the starch granule is composed mainly of low-molecular-weight (LMW) compounds and high molecular weight carbohydrates. Within the former, no sugars, proteins or peptides were found (Seguchi, 1995) making the phospholipids its main component together with the HMW carbohydrates responsible for maintenance of the wheat starch granule structure. The role of lipids is referred as of special importance. Although the discussion on the role of phospholipids in compactness and stability of starch granule carries on, the opinion is that internal phospholipids seem to be a template for amylose helix structure (Seguchi & Kanenaga, 1998). The presence or absence of V-type structure using the X-ray diffraction can confirm or eliminate the lipid–amylose complexes within the granule. Internal and external lipids could influence the starch properties such as temperature of gelatinisation, power of swelling, viscosity and leaching of soluble carbohydrates (Kaukovirta-Norja, Reinikainen, Olkku, & Laakso, 1997; Kitahara, Tanaka, Suganuma, & Nagahama, 1997; Lin & Czuchajowska, 1998). Especially the amylose–lipid complexes have been studied due to their influence on the starch behaviour during dough making or baking. Seguchi and Matsuki (1977) were reported the importance of non-starch compounds associated with the wheat starch granule surface in improving the cake texture, e.g. the degree of springiness and gumminess.

Taking into consideration mentioned above role of the external and internal structure as well as surface components of starch granule in creation of their properties responsible for properties of food and still being under discussion problems, in this work much effort has been directed towards:

- identification of the chemical nature of non-starch compounds present on wheat, corn and potato starch granule surface with special reference to lipid and carbohydrate fractions,
- characterization of the skeletal material of starch granules after their stepwise extraction with sodium dodecyl sulfate and mercapthoethanol, with special attention paid to crystallinity of the granule and molecular weight of skeletal part,
- visualization of the effect of extraction procedure used on distribution of amylose and amylopectin within the granule when heated in water at gelatinisation temperature by means of light microscopy (LM).

2. Experimental

2.1. Material

The analyzed material was the commercial wheat (Roquet), corn (Cerestar) and potato (Superior Standard) starches. The material was obtained from Starch and Potato Products Research Laboratory in Luboń.

2.2. The preliminary chemical composition of starches

The nitrogen, moisture, ash and lipids contents were determined according to Kjeldahl, AAAC 44-40 (AACC, 1983) and PrPN -EN ISO 3593 and Kaukovirta-Norja et al. (1997) methods, respectively.

The amylose content was assayed by the method of Moririson and Laignelet (1983).

The extraction of starch granule lipids was performed by the methods described by Kaukovirta-Norja et al. (1997). According to these authors and Morrison 1998 the term ‘surface lipids’ is used in this article in order to describe the lipid fraction extracted by cold solvent (*n*-propanol-water mixture 3:1 v/v at 20 °C for 30 min using the following ratio: 20 mL of solvent/2 g of starch) from the granule surface; whereas lipids removed by a hot solvent also from the interior of the granule were counted as total lipids. The identification of chemical nature of the obtained lipid fractions was performed using GC techniques according to Vasanthan and Hoover (1992) after conversion of fatty acids to methyl esters and their identification on the basis of retention times of samples and standards in a Varian 3400 Autosampler gas chromatograph with capillary column 30 m × 0.32 mm × 0.25 μm (model high performance capillary column 19091N-113, HP-INNOWax/crosslinked polyethylene glycol, Agilent Technology). The flow rate of helium carrier gas was 30 ml/min. The results were expressed as the sum of fractions of different saturation.

2.3. Preparation of Remazolbrilliant Blue-starch complex (RBB-starch) and its extraction with SDS/ME

Starch (12 g) was suspended in 6 ml of water, and the aqueous solution of Remazolbrilliant Blue R (RBB) dye (1.2 g/120 ml) was added to the suspension. During 45 min of continuous shaking (120 rpm) of RBB and starch mixture in water bath at 25 °C, sodium sulfate (24 g) was added in

several portions. Afterwards, the reaction mixture was treated with a solution of trisodium phosphate (1.2 g/12 ml), and the shaking was continued for 75 min. The mixture was centrifuged and the supernatant was discarded. The treated starch was washed with water until the blue colour in supernatant disappeared and then freeze-dried. The obtained RBB-starch complex was washed with aqueous 1% SDS containing 1% 2-mercaptoethanol (ME) by gentle agitation at room temperature for 14.5 h (for that complete procedure a term of 'onefold extraction' is used) and centrifuged (1.700 × g, 20 min). Supernatant was collected, dialysed against water, freeze-dried and the remaining RBB-starch (pellet) after freeze drying continued to be fivefold, and fifteenfold extracted using each step of the procedure described above (e.g. washing with SDS/ME, agitation for 14.5 h, centrifugation, supernatant collection, dialysis and freeze drying). After each single procedure of extraction, the pellet was again resuspended in fresh SDS/ME (Seguchi & Kanenaga, 1997).

After five- and fifteenfold extraction with SDS/ME, the residual part of starch (remainings of the pellet) was washed several times in distilled water, centrifuged (2000 rpm × 10 min) and dried at room temperature. So obtained material was used for further analyses (LM microscopy, column chromatography, and X-ray investigations).

2.4. Sepharose CL-2B size-exclusion chromatography

For the analysis of the molecular structure, disruption of the granular arrangements followed by complete dissolution of the starch chains in a proper medium is a prerequisite. Dimethyl sulfoxide (DMSO) as well as other aqueous solutions of inorganic alkali are commonly used as starch dissolution solvents. The thermal treatment of the starch granules in DMSO resulted in their complete dispersion. That fact is probably connected with an effective break of the intramolecular bonds between starch chains (You & Lim, 2000) during heating of the starch granules in DMSO.

The freeze-dried SDS/ME extract (supernatant) was dissolved in 1 ml of 90% DMSO by heating for 3 min at 100 °C. After filtration (0.4 μm) the sample was sonificated and subjected to Sepharose CL-2B (1.0 × 95 cm) size-exclusion chromatography. The absorbance at 650 nm for RBB (Seguchi & Kanenaga, 1997) as well as the absorbance at 490 nm for total carbohydrate (Dubois et al. 1956) was measured. The profiles were obtained by expressing the measured absorbance values as optical density (absorbance value × 4 ml of each of collected fractions). Molecular mass (up to 788000) was determined using the Pullulan Shodex STANDARD P-82 (Showa Denko K.K.), habitually used for this kind of analysis (Seguchi & Kanenaga, 1997).

The low molecular weight (LMW) material was collected only so as to characterize its chemical character.

2.5. Purification of LMW material and NMR analysis

The LMW material was purified by the preparative thin layer chromatography on silica gel plates (Merck). A mixture of: CH_2Cl_2: MeOH: H_2O (65:25:4 v/v/v) was used for plate development. A mixture of CH_3COOH: H_2O: H_2SO_4 (20:4:1, v/v/v) and UV light were used for detection of bands. The bands were eluted and collected after evaporation of the solvents. The purified material was used to run the ^{13}C NMR and ^{1}H NMR spectra.

The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Unity spectrometer operating at 500 and 125.7 MHz, respectively, using CD_3OD as a solvent at 30 °C. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired with standard conditions. The COSY 2D NMR spectra were acquired in the phase-sensitive mode. Data were collected in 1024 × 256 matrix with a spectral width of 5000 Hz and a 2 s recycle delay and processed in a 1024 × 1024 matrix. (Jimeno, Valverde, Błaszczak, Fornal, & Amarowicz, 2002).

2.6. X-ray diffraction investigation of residual RBB-starch

The X-ray diffraction patterns were obtained from untreated starches (wheat, corn, and potato) as well as fifteenfold extracted one. 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 $\theta = 2°–18°$ (Lewandowicz, Jankowski, & Fornal, 2000).

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 untreated and residual starches were expressed through the mathematical calculation of peak area under the curve in X-ray patterns (Nara, Mori, & Komiya, 1978) of the analysed starches using Micro Image 4.0 for Windows (Olympus Optical co. Europe).

2.7. Light microscopy (LM)

The extraction was monitored by microscopic investigation, using different LM techniques: staining with iodine, Nomarski contrast and polarized light.

RBB-starch pellet (0.8 g) resuspended in water (10 ml) was heated for 15 min at 75 °C for cereal starches (wheat, corn) and at 65 °C for potato starch. The above temperatures were selected as the points where gelatinisation process begins. Starch pastes were smeared over a microscopic glass and after cooling stained with iodine. For Nomarski and polarized light, the residual starch was suspended in the drop of water and covered by cover slip. The preparations

Table 1
The preliminary chemical composition of starches

Starch	Nitrogen (mg N/g d.m.)	Ash (mg/g d.m.)	Moisture (%)
Wheat	0.493	0.207	12.2
Corn	0.690	0.077	11.9
Potato	0.091	0.315	16.4

were viewed and photographed in a LM microscope OLYMPUS BX60.

3. Results and discussion

3.1. Chemical composition of surface starch material

It was found that chromatography profiles of onefold extracted sodium dodecyl sulfate SDS/ME extracts obtained from wheat, corn and potato starches differed from each other in optical density values. The differences in shape of the obtained profiles between potato and cereal starches were also observed, what seemed to be rather expected bearing in mind the results of preliminary characteristic of the chemical composition of the starches used in this study (Tables 1 and 2).

The A_{490} profile of cereal starches showed the presence of high-molecular (HMW) as well as low-molecular (LMW) surface compounds (Fig. 1a,b). However, the HMW (800–200 kDa) fraction was rather small compared to the LMW material (100–120 kDa), which was characterised by much higher optical density value. The A_{650} profiles of non-starch compounds extracted from the surface of wheat and cornstarches indicated RBB-non-starch compounds complex. That complex has been already known from previous analyses made by Seguchi (1995) and Seguchi and Kanenaga (1997, 1998), however only in the case of wheat starch. These authors identified lysophosphatidylglycerol (LPG) as the main LMW non-starch material (Seguchi & Kanenaga, 1998), and they showed that SDS/ME solution solubilized starch polymers from the outside to the inside

Table 2
Characteristics of the starch granule lipids (W—wheat, C—corn, P—potato)

Composition of extracted lipids (%)						
Lipids	Surface lipids (cold solvent extraction)			Total lipids (hot solvent extraction)		
	58.8 W	12.1 C	9.9 P	100 W	100 C	100 P
Saturated	35.73	37.36	42.08	46.99	41.76	59.39
Mono-unsaturated	24.93	9.56	11.23	12.60	11.08	19.41
Poly-unsaturated	39.34	53.08	46.69	40.41	47.16	21.20

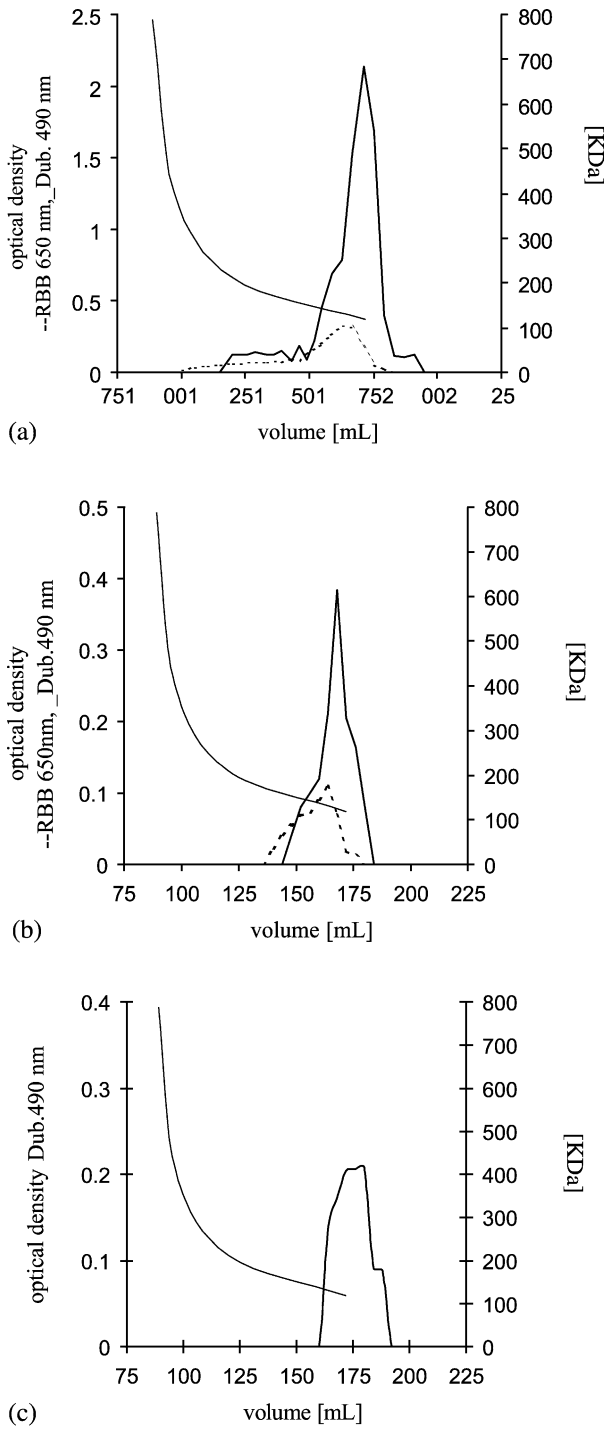

Fig. 1. Sepharose CL-2B gel-filtration chromatography profile of non-starch compounds onefold extracted with SDS/Me from the starch granule surface: (a)wheat, (b)corn and (c)potato starch.

of granules resulting in extraction of the LPG-RBB complex. In opposite to these authors, a lower optical density value related to HMW material was found in the analysed cereal starch extracts (Fig. 1a,b). This fact could indicate that the concentration of HMW compounds was lower as it was shown by Seguchi and Kanenaga (1997). Similarly, the concentration of LMW material also

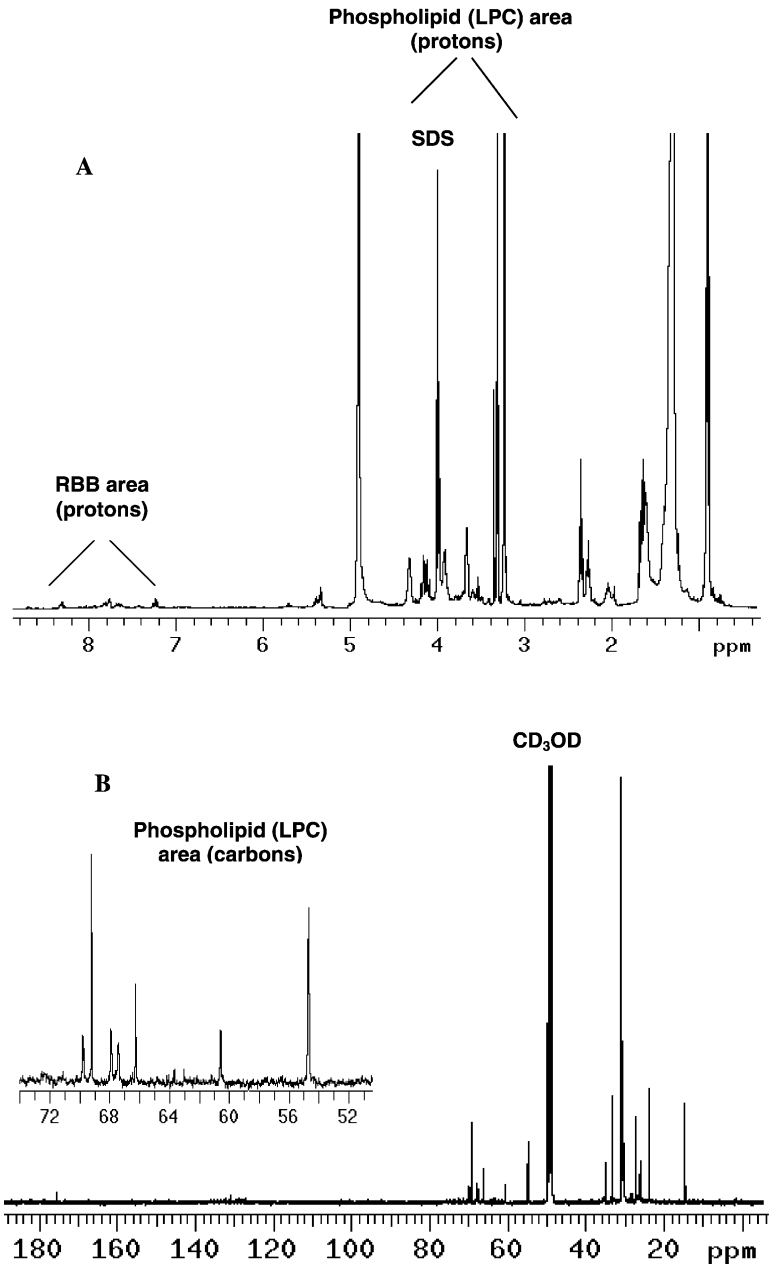

Fig. 2. A—500 MHz ^{1}H spectum and B—125 MHz ^{13}C spectra of of LMW material onefold extracted (SDS/ME) from wheat starch granules.

seemed to be lower in wheat and cornstarch extracts compared to previously published data (Seguchi & Kanenaga, 1997).

In the case of potato starch, no RBB-non-starch compounds complex was found. The potato starch extract consisted of soluble carbohydrates (120 kDa), only (Fig. 1c). The chromatographs showed that onefold extraction of wheat, corn and potato starch granules with SDS/ME caused elution mainly of the LMW surface material, and only in the case of cereal starches the presence of HMW material was found.

The ^{13}C and ^{1}H NMR spectroscopy analyses were performed so as to obtain more detailed information about the LMW material characterized in A_{650} chromatography profile of wheat starch. The ^{13}C NMR spectrum of purified LMW material showed strong peaks corresponding to phospholipid carbons (52–72 ppm) and acyl carbons (15–40 ppm) resonances (Fig. 2b). The ^{1}H NMR spectrum of analysed LMW material also showed the multiplets in two different regions, 3.0–4.5 ppm corresponding to phospholipid protons and 0.8–2.6 ppm corresponding to acyl protons (Fig. 2a). On the basis of series of selective experiments, at least three major components can be identified in ^{1}H NMR spectrum of LMW material: an intense multiplet at 4.0 ppm and some of the acyl multiplets are due to SDS,

the singlet at 3.23 ppm, the remaining multiplets between 3.0–4.5 ppm and the corresponding acyl protons related to phospholipid (which was assigned to lysophosphatidylcholine (LPC) in our separate study (Jimeno et al., 2002)), and the weak signals present in aromatic region at the 7.5–8.4 ppm are due to trace amounts of RBB.

3.2. Chemical composition of starch surface lipids

The amount of lipids extracted from starch granules with solvent mixture: *n*-propanol: water (3:1, v/v) was dependent on the temperature of extraction used (cold or hot solvent), whereas the chemical composition of removed material was related to botanical origin of starch (Table 2). Data presented in Table 2 indicate that the *n*-propanol starch extracts were composed of saturated, mono-unsaturated and poly-unsaturated lipids in proportions differing for wheat, corn, and potato starch. A hot-solvent extraction resulted in considerable changes of the amount of extracted lipids between analyzed starches and significantly influenced the ratio of lipid fractions in the starch extracts. The highest amount of extracted surface lipids with cold solvent was found in the wheat starch and they occupied as much as 58.8% of total lipids extracted. The lowest amount of that fraction was found in potato starch. Internal lipids were calculated by the differences between hot and cold extracts and they were found to be the main component of corn and potato starch, occupying as much as 87.9 and 90.1% of total extracted lipids, respectively. These results, although obtained by different method of extraction, seem to confirm those presented by Vasanthan and Hoover (1992) who also found an increase in lipid compounds in extracts after a hot-solvent treatment of starches from different botanical sources. The sequential method (cold and afterwards hot) of extraction used, increased the efficiency of extracted starch material especially from the interior of the granules (16.2%—for potato starch and 94.8%—for corn one). According to mentioned-above authors the obtained results were not related to those of Milligan and Morrison, who are of the opinion that potato and legume starches were devoid of internal starch lipids. Such a discrepancy in quantity and quality of lipid compounds from extracted starch reported by different authors could result not only from different methods used in lipid extraction but also from differentiation in the granular starch structure and different chemical character which in turn strongly influences properties of starch.

3.3. Microscopic investigation of skeletal structure of starch granule

The differences in chemical character between the components of starch granules soluble and insoluble in SDS/ME, appearing during repeated extraction led to total elution of the soluble material from granules. The data summarized in Table 3 show that the amount of SDS/ME soluble material in starch (expressed as the amount of amylose) decreased consequently with stepwise washing of the starch granule.

Table 3
Changes in the amylose content in residual starch (pellet) during washing with SDS/ME

Starch	Amylose content (%)		
	Steps of starch washing		
	Untreated	After 5th	After 15th
Wheat	22.2 ± 0.3	14.4 ± 0.2	1.9 ± 0.1
Corn	24.3 ± 0.3	11.9 ± 0.1	0.9 ± 0.1
Potato	20.0 ± 0.2	18.3 ± 0.3	4.0 ± 0.1

± —Standard deviation.

The LM pictures of wheat, corn and potato starch pastes (heated at 75 °C for cereal and at 65 °C for tuber) after fivefold and fifteenfold granules extraction are presented in Figs. 6 and 7. It is clearly visible that removing of non-starch surface compounds resulted in significant changes in granules. Strongly swollen, hollow and dimpled shape of wheat starch granules was observed (Fig. 6a) during their heating at gelatinization temperature already after fivefold extraction. In opposite to wheat, staining blue due to amylose present in the granule, cornstarch granules heated at the same conditions, showed distinct pores and channels within the granules being in some cases cracked. Progressive fragmentation of cornstarch granules into several parts/segments is also evident (Fig. 6c). That fact could suggest some heterogeneity within the granule structure, with some resistant and more open—accessible for solvent treatment regions (Leach & Schoch, 1962; Seguchi & Kanenaga, 1997; Vilwock, Eliasson, Silverio, & Bemiller, 1999). The observed channels and pores as well as cracks caused slow but effective elution of soluble starch material in SDS/ME (Table 3), what in turn could result in the beige appearance of starch granule after iodine staining. The microscopic picture of potato starch (Fig. 6e) differs from cornstarch mainly in the diameter of the swollen granules, being much higher. Most of the potato granules, as in the case of corn ones, stained red-brown with iodine. Additionally, in the liquid phase of the paste some pale-blue colour is visible. Obanni and BeMiller (1996) reported that these observations could result from some disproportion of starch structure, mainly in amylopectin (staining brown), and amylose (staining blue) ratio. These findings seem to be in close relation to the data presented in Table 3, as well as to the photos taken after fifteenfold extraction

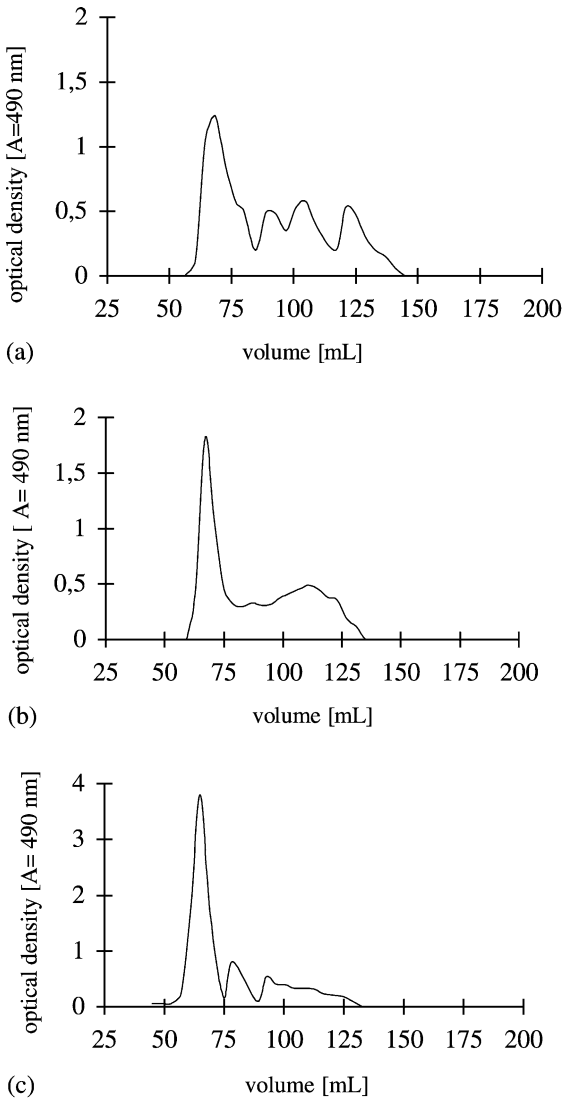

Fig. 3. Sepharose CL-2B gel-filtration chromatography profiles of molecular mass distribution of RBB-starch pellet fifteenfold extracted with SDS/Me: (a)wheat, (b)corn, (c)potato.

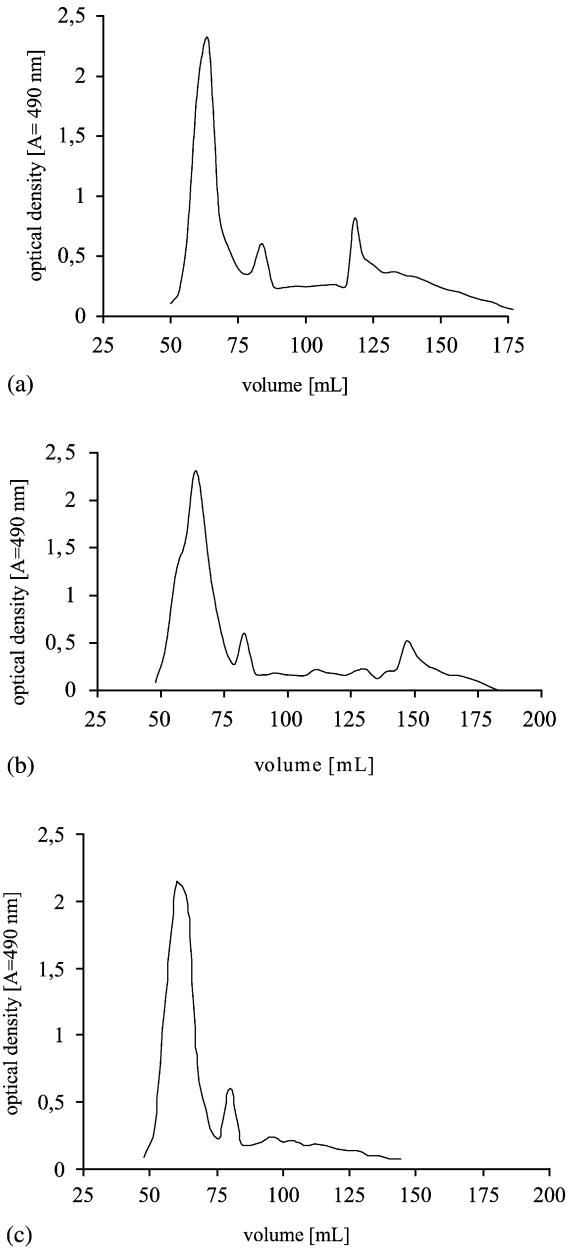

Fig. 4. Sepharose CL-2B gel-filtration chromatography profiles of molecular mass distribution of native starches: (a)wheat, (b)corn, (c)potato.

(Fig. 6b,d,f). The most striking difference is the absence of amylose in wheat starch, while in the potato pellet the liquid phase of the paste still contains blue phase of amylose (Fig. 6f). The granules of all analysed starches became somewhat ghost-like. On the basis of the changes observed in the starch structure during extraction, it could be suggested that the beige-pale coloured starch granules consist mainly of insoluble in SDS/ME material, which formed the skeletal structure of granules.

The chromatography profiles (Fig. 3) of residual starch material (wheat, corn, and potato), obtained after fifteenfold extraction were in close relation to the above-presented microscopic analysis. The profiles of wheat and corn-starches fifteenfold extracted showed significant changes in molecular mass distribution compared to the untreated ones (Fig. 4). However, the changes seem to be connected only with an amorphous part of starch, i.e. with amylose. The peak relating to high molecular material, which can be observed in chromatography profiles of the extracted starches probably corresponded to unchanged amylopectin.

The profile of molecular mass distribution of residual potato starch after fifteenfold extraction seemed to be slightly changed compared to untreated potato starch, and it was different from the above-described profiles of cereal starches. The curve of molecular mass distribution of residual potato starch still consisted of two distinct peaks—the first corresponding to amylopectin, and the second related to the molecular weight of amylose. Moreover the identified peaks of amylopectin and amylose appeared in

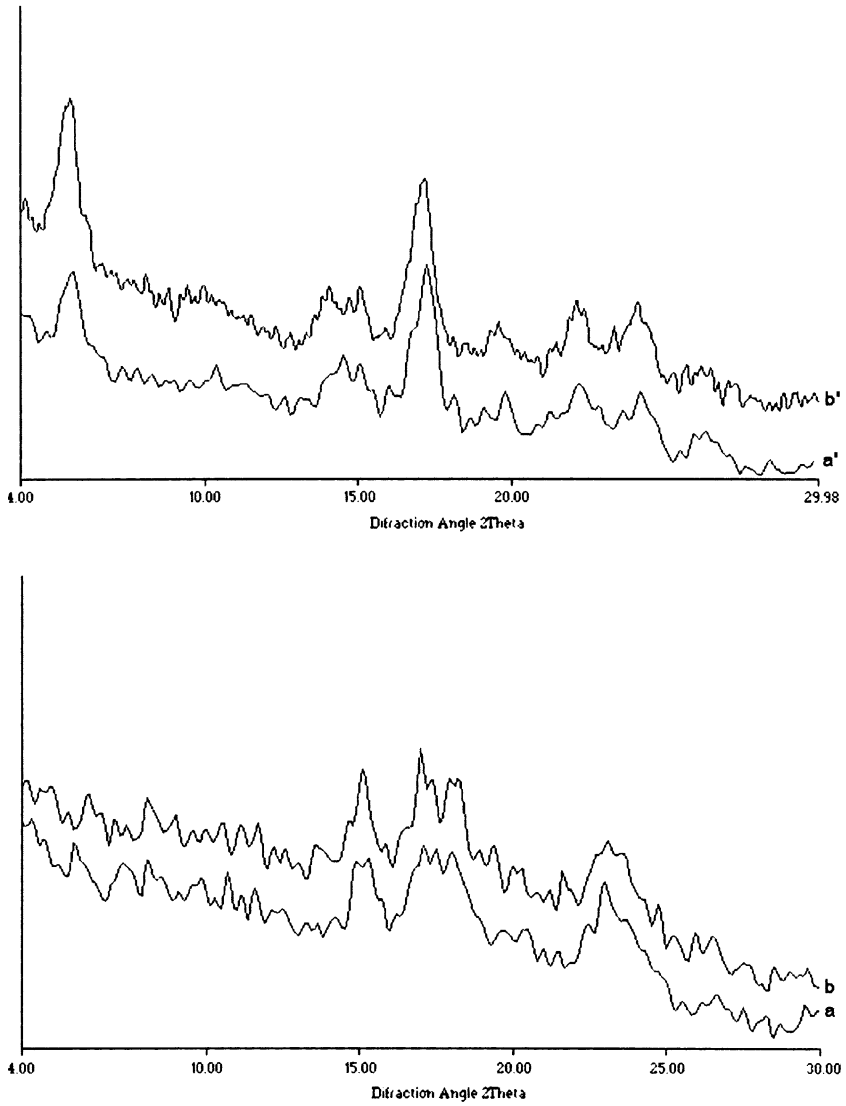

Fig. 5. X-ray diffraction patterns of starches: (a)native wheat and (a′) potato, (b)wheat and (b′) potato RBB-starch pellet fifteenfold extracted.

the profile of residual potato starch at the same retention volume compared to native potato starch.

The X-ray patterns confirmed A-type for wheat and B-type crystallinity for potato starch. Crystallographic profiles in all analysed starches (fifteenfold extracted) seemed to retain the type of crystallinity of the untreated ones (Fig. 5). However, the detailed analysis of X-ray profiles of residual starches indicated that SDS/ME extraction of non-starch compounds as well as removal of soluble starch material influenced the relative crystallinity (untreated to residual starch) of starch granule. The digital data showed an increase of relative crystallinity of residual potato and wheat starches, i.e. 23 and, 18, respectively compared to crystallinity of native starches.

When the fifteenfold extracted starch granules were viewed under Nomarski contrast, they showed only slight changes in the starch structure (Fig. 7). The crystallinity of those starches also was visualized under polarized light. Most of starch granules still maintain birefringerency. The presented results allowed to put forward a hypothesis that it is mainly amylopectin that forms the skeletal structure of starch as well as that progressive elution of starch material (soluble in SDS/ME) does not affect changes in the type of starch crystallinity.

It was expected that prolonged time of starch-RBB complex extraction with SDS/ME would correspond with the depth of solvent penetration and stepwise opening of the inner starch granule strata. Opposite to Seguchi and Kanenaga (1997), who showed opened starch granules using shearing forces (although not defined by number), in our work we would like to validate how gentle agitation can open the starch granule lamellar structure. The microscopic investigations showed that the forces used were not of shearing character and did not open the starch granule interior. It was also evident that distortion

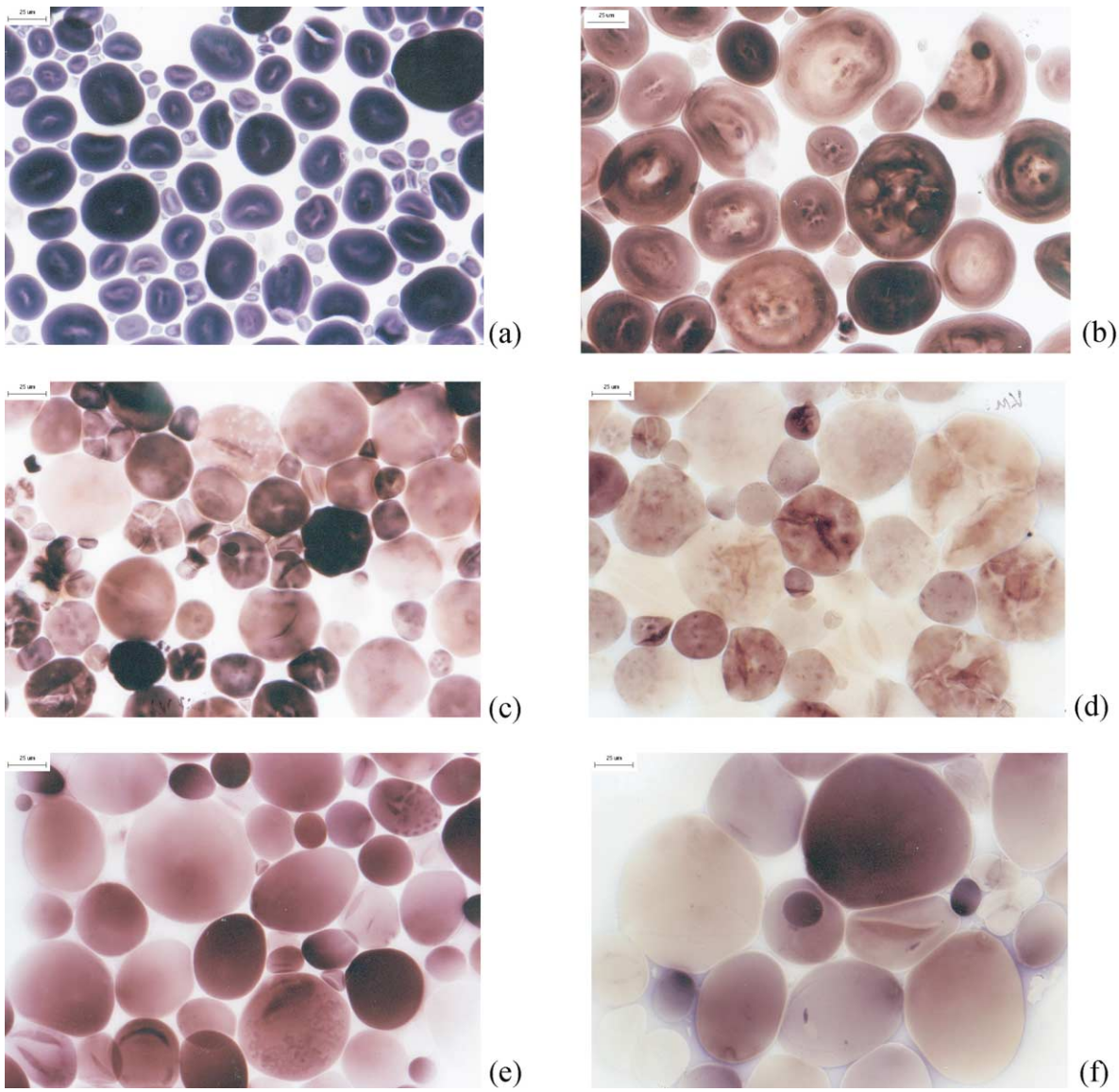

Fig. 6. Photomicrographs (LM) of starch pastes cooked at 75 °C for wheat and cornstarch and at 65 °C for potato starch.: (a) wheat starch five and (b) fifteenfold extracted with SDS/ME; (c) cornstarch fivefold and (d) fifteenfold extracted with SDS/ME; (e) potato starch fivefold and (f) fifteenfold extracted with SDS/ME.

of granule integrity by shearing forces was the main reason of continuous leakage of the soluble starch material during its extraction. However, it was also found that even fifteenfold extraction with SDS/ME did not guarantee a complete elution of the whole soluble material. It was also noted in the study of Atkin et al. (1999) that not all amylose could be separated completely from the amylopectin. Some amylose present within amylopectin crystallites might be difficult to remove as it becomes trapped between packing ordered amylopectin structures. Thus it is possible that the skeletal structure of residual starch could be mostly composed of amylopectin having also a wide range of high molecular mass fragments (Fig. 3). LM pictures showing the granules mainly stained beige-brown could also confirm this fact. All the above-mentioned changes did not result significantly in X-ray diffraction pattern as it was reported by Seguchi (1995). Gentle extraction removing LMW and HMW carbohydrates without granule destruction has did not change the type crystallinity, however, has to some extent an influence on their relative crystallinity. Also that finding being opposite to Seguchi, who found no crystallinity after five days of SDS/ME extraction, confirms that also physical disorganization of the granule (central fragment can fall out during extraction with prolonged stirring). Therefore, while speaking about the internal 3D structure of the starch granule the differentiation between the role of chemical and physical factors should be discussed separately.

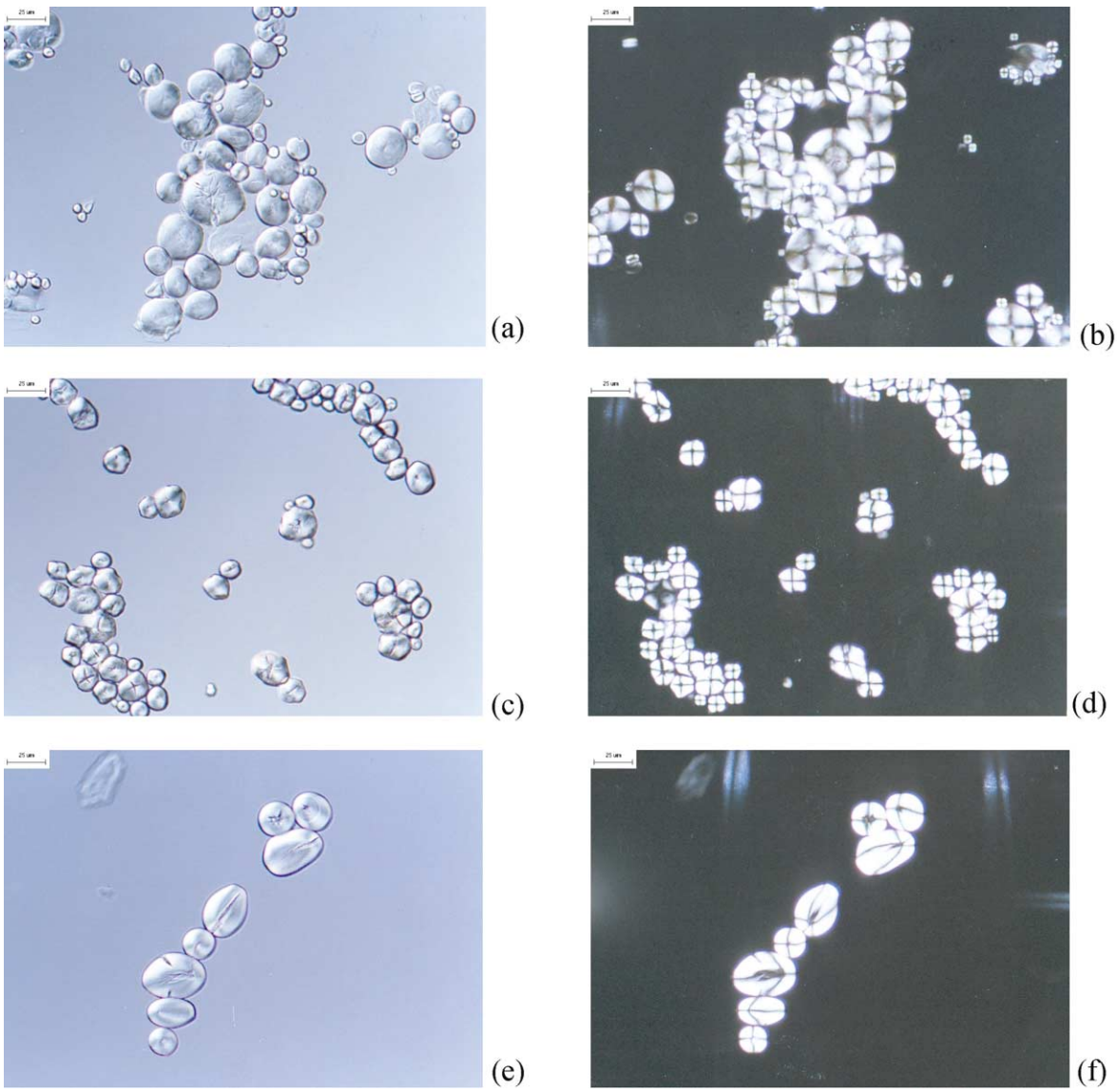

Fig. 7. Photomicrographs (LM) of water starch suspension after fifteenfold extraction of non-starch compounds with SDS/ME: (a)Nomarski contrast, (b) polarised light—wheat; (c)Nomarski contrast, (d) polarised light—corn; (e)Nomarski contrast, (f) polarised light—potato.

4. Conclusions

The LMW compounds were the main components of onefold extracted starch extracts and only in the extracts of cereal starches some HMW non-starch compounds were found. The lysophosphatidylocholine (LPC) was found to be the main non-carbohydrate LMW product of wheat starch.

The amylopectin was found to be the main component of residual (i.e. after fifteenfold extraction) starch. The amylopectin, which was insoluble in SDS/ME, created the skeletal structure of all analysed starches.

The extraction of non-starch compounds as well as the progressive elution of soluble starch material (amylose) did not affect changes in type crystallinity of the starch granules but influenced the relative crystallinity of residual starches.

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