

Chemical-mechanical treatment of potato starch for isolation of nanocrystal particles

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Abstract

Starch nanocrystals were isolated by a combination of acid hydrolysis and sonication of native potato starch. The obtained samples were examined by XRD, scanning electron microscopy and static light scattering. An increase in hydrolysis time increases the degree of crystallinity from 47 to 66%. By changing the processing conditions, it is possible to isolate particles of a given size. First, submicron flat particles with dimensions of 3000–5000 nm and a thickness of 80–100 nm are obtained. With longer processing, approximately isometric nanoscale particles remain. Based on the obtained data, a stepwise topochemical mechanism for the formation of starch nanoparticles was proposed.

Keywords

starch nanocrystals
potato starch
ultrasonic treatment
chemical-mechanical treatment
topochemical hydrolysis of starch granule

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Key findings

- The process of acid hydrolysis of potato starch has a pronounced topochemical nature.
- Ultrasonication of crystalline starch nanocrystals results in particle size reduction.

1. Introduction

Among the numerous practical applications of nanoparticles from natural polymers, perhaps, the most known is their use for reinforcement of biodegradable materials [1–3]. No less interesting is the use of nanosized particles from natural biopolymers as adjuvants.

Adjuvants are inorganic and organic nanoparticles that are able to adsorb and retain antigens on their surface for a long time, which increases the duration of their effect on the body's immune system [4–7]. Inorganic and organic adjuvants based on synthetic and natural polymers have been used. Inorganic adjuvants based on oxide and aluminum hydroxide nanoparticles are easy to prepare, and their surface is easy to modify. However, they often show cytotoxicity, have abrasive properties, are unfriendly to living organisms and have a short sedimentation time, which makes their use in vaccination inconvenient. Nanoparticles of natural polymers in general and starch nanoparticles in particular are non-toxic, biodegradable; and biocompatible, their surfaces can be easily modified [8]. Moreover, the sedimentation time of the SNP-based adjuvants is long enough that there is no need to shake contin-

uously the needle vaccinators during vaccination. According to the literature, the following polymer-based nanomaterials are used as adjuvants: starch, cellulose, chitosan or some alginic acid salts – alginates. We chose potato starch, which can most easily be extracted from biomass and which, unlike cereal starches, contains significantly less fat and protein facilitating the purification process.

2. Materials and methods

2.1. Materials

For this work, normal potato starch was isolated from Rosara breed, and then it was dried at room temperature [10]. The selected breed did not belong to highly starchy or waxy potato, had about 14% starch and the amylose and amylopectin contents were assumed to be approximately 30 and 70%, respectively. The moisture and protein contents of potato starch were 12.4% and 0.07%, respectively.

2.2. Preparation of SNC

Starch nanoparticles were isolated according to the 'rapid method' using diluted sulfuric acid [7–8]. Starch (133 g, dry basis) was suspended in 500 mL of H₂SO₄ (3 M) and

incubated with 500 rpm mechanical stirring at 37 °C for 1–6 days. The resulting suspension was washed by successive centrifugation (3500 g, 10 min) with distilled water until neutral pH had been reached; then the precipitated residue was washed with ethanol (95%) and dried in air at room temperature. The yield of hydrolysis (Y) was calculated as the ratio of the weight of solid starch residues to the dry weight of the initial starch. Therefore, the degree of hydrolysis refers to $1 - Y$ (%).

$$Y(\%) = \frac{\text{the weight of solid starch residues}(g)}{\text{weight of initial starch}(g)} \quad (1)$$

The resulting SNC suspension was subjected to ultrasonic treatment with 400 W power for 20 seconds, 5 times, with cooling in an ice bath.

2.3. Morphology observation

The morphology of the native and hydrolyzed starches was observed using a field emission scanning electron microscope (Hitachi TM 1000, Japan) at an acceleration voltage of 15 kV. A drop of suspension was spread onto a carbon tape and dried under vacuum. The samples were then coated with a gold layer by ion sputtering using an auto fine coater JFC-1600 (JEOL, Japan).

2.4. Size distribution analysis

Particle size distribution (PSD) of the samples was carried out at 25 °C with a Laser particle size analyzer (SALD-7500, Shimadzu, Japan). The pH of the suspensions was approximately adjusted to 7. A value of 1.40 was considered as the refractive index, and the data were means of three replicates.

2.5. XRD analysis

X-ray diffraction patterns of the investigated starches were recorded using Cu K α radiation on a D8 Advance X-ray diffraction meter (Bruker AXS, Germany) with Bragg-Brentano geometry equipped with a one-dimensional Lynx-Eye detector and a K β filter. PXRD patterns were collected in the interval $5^\circ < 2\theta < 40^\circ$ with a step size of $\Delta 2\theta = 0.0195^\circ$ and a counting time of one second per step. The relative crystallinity was calculated by estimating the percentage of the crystalline area relative to the total diffraction area according to Nara and Komiya [9].

3. Results and discussion

3.1. Size distribution

Figure 1 shows the size distribution for the native potato starch. The starch is characterized by a bimodal distribution, 5% of all particles have diameters from 1 to 10 μm , and 95% from 10 to 150 μm . Hydrolysis for 6 days leads to the disappearance of a fraction $> 50 \mu\text{m}$ in size. The volume fraction of particles up to 10 μm in diameter increased from 6% to 65%. The impact of ultrasonic treatment of hydrolyzed starch leads to the appearance of a nanosized fraction of starch 20–350 nm, which constitutes 57% of the total mass of particles in suspension. This observation shows that acid hydrolysis leads to the destruction of native starch granules. However, to obtain colloidal solutions of starch nanocrystals, mechanical processing is necessary.

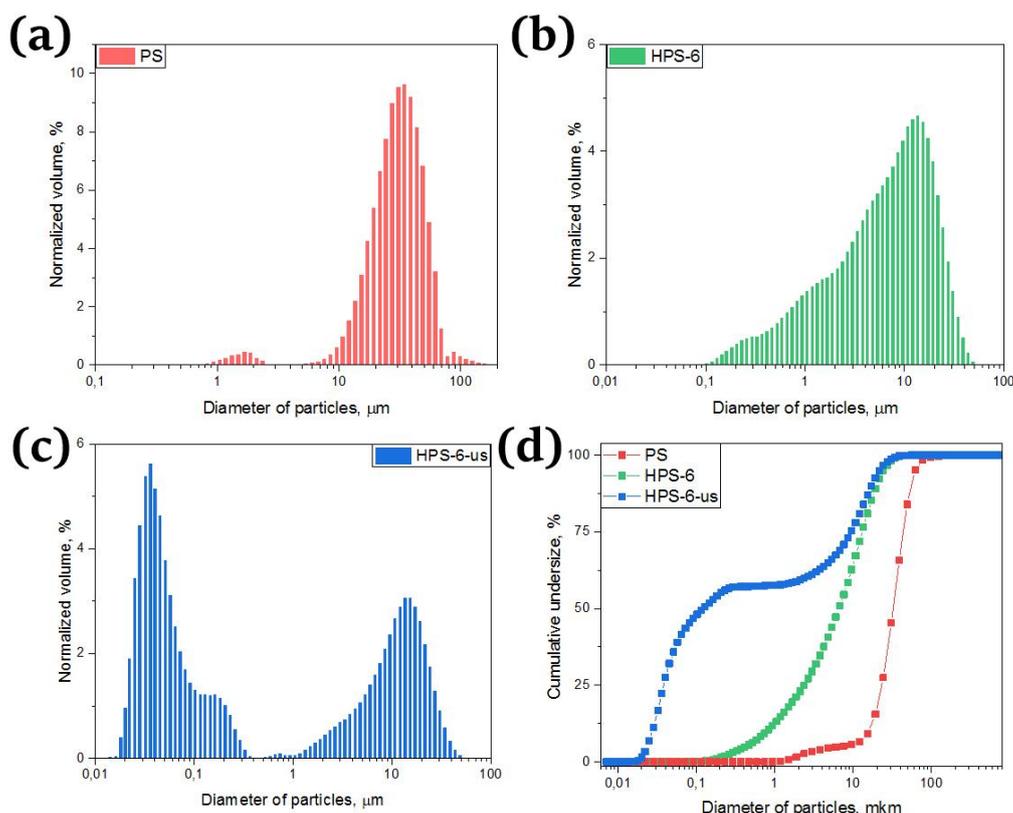


Figure 1 Particle size distribution of samples of native potato starch (PS, a), obtained after 6 days of hydrolysis (HPS-6, b) and hydrolyzed starch subjected to ultrasonic treatment (HPS-6-us, c). The integral form of these distributions (d).

3.2. Crystalline structure of initial and hydrolyzed potato starch

Figure 2 shows X-ray diffraction patterns of the native and hydrolyzed starch. The native potato starch possesses a distinct B-type crystalline structure with diffraction peaks at 5.6° , 11.4° , 14.4° , 15.0° , 17.2° , 19.7° , 22.2° , and 24.0° . It is well established in the literature that in the acid hydrolysis the amorphous regions of starch granules are the first to react. Thus, diffuse scattering from disordered phase supposed to be decreased and the degree of crystallinity – increased during the acid treatment. Crystalline indices are presented in Table 1.

3.3. Peculiarities of acid hydrolysis of potato starch

The degree of acid hydrolysis was measured by the weight of the dry residue contained in a daily taken aliquot of the reaction mixture (see 2.2). The hydrolysis of starch grains is typically represented by two processes having different rates. The initial hydrolysis step is slow and involves hydrolysis of the crystalline regions of the outer layer. Microscopic observations carried out during the six-day hydrolysis showed that the size and morphology of starch grains remained virtually unchanged. Due to the tight packing of the double helices, there is limited penetration of H_3O^+ ions in the crystalline regions. The second hydrolysis step corresponds to hydrolysis of amorphous regions. It proceeds quickly, leads to a noticeable loss of starch mass and a change in its morphology. In the first 24 hours of slow hydrolysis, the mass loss was about 10%, after 70–84 hours of hydrolysis the mass loss was 70–80%.

Microscopic observations show that a significant part of the starch granules is deformed and split into pieces (Figure 3b and 3c).

Table 1 Index of crystallinity of native and hydrolyzed starch.

Type of samples	PS	HPS-3	HPS-6
Crystallinity, %	47 ± 2	59 ± 2	66 ± 2

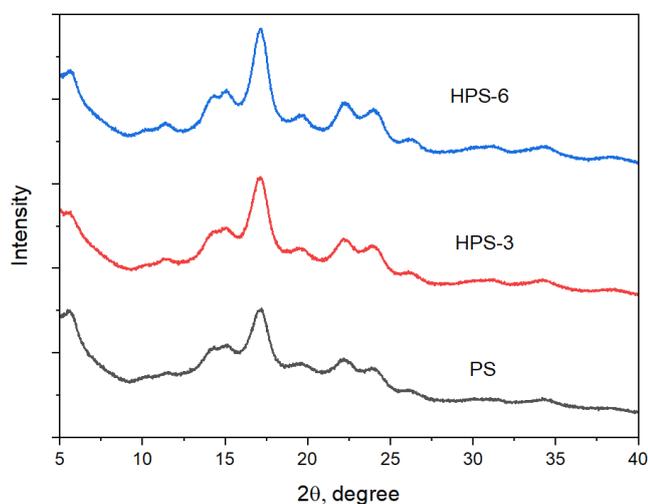


Figure 2 XRD patterns of native potato starch (PS), starch hydrolyzed for 3 days (HPS-3) and for 6 days (HPS-6).

After three days of hydrolysis, many outer layered shells of granules, "ghost granules", are observed in the samples, which indicates their increased resistance compared to the inner layers. Similar observations were made in earlier papers [11]. It is also worth noting that on the third day of hydrolysis, the samples contain particles of varying degrees of destruction, from whole spherical granules (outwardly close to native starch) to submicron particles. Therefore, we hypothesize that native starch granules can vary greatly in their resistance to acid hydrolysis depending on their size and the presence of internal defects such as cracks and cavities [12]. The different reactivity of starch granules leads to the need to increase the hydrolysis time, which leads to low yields of nanoparticles [13]. A possible solution to this drawback may be the mechanical activation of starch grains at various stages of hydrolysis.

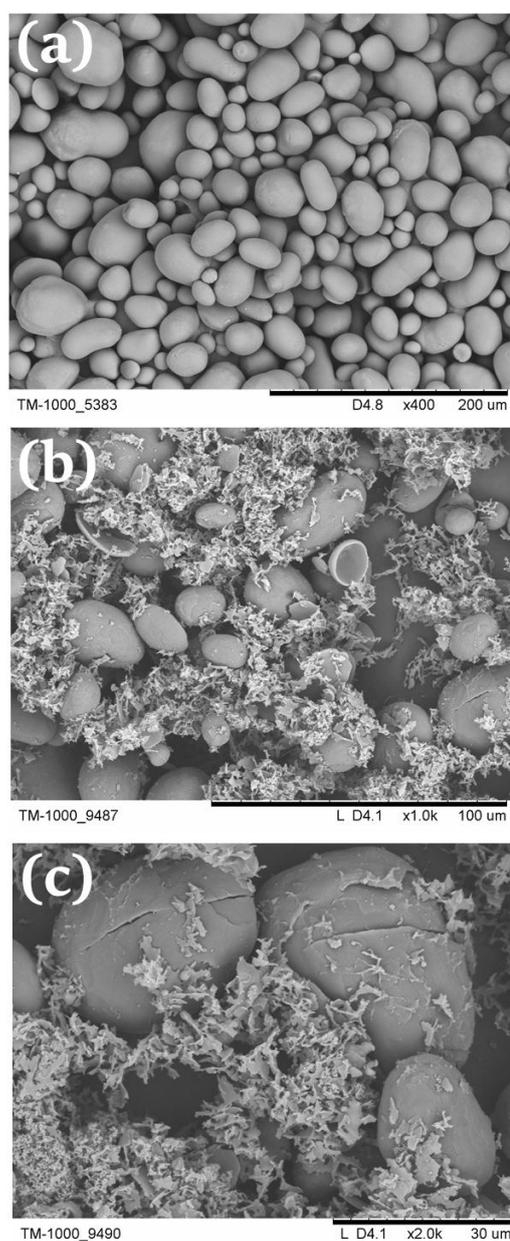


Figure 3 Initial starch granules before hydrolysis (a), starch granules after 6 days of hydrolysis at different magnifications (b, c).

3.4. Topochemical mechanism of starch granule hydrolysis

The data, represented in micrographs in Figure 4, help to understand the topochemical mechanism of starch grain hydrolysis. Topochemistry is a branch of chemistry that studies solid-phase reactions occurring locally in certain areas of a solid [14]. The local nature of the reaction is due to the increased reactivity of the solid at a given site and to the large free volume, which ensures the mobility of the reactants and reaction products. Starch grains consist of alternating amorphous and semi-crystalline layers. In amorphous areas of starch, consisting mainly of amylose, the necessary conditions for the topochemical development of reactions are fulfilled, at which the rate of hydrolysis in the center of the grain is so high as to observe empty and semi-empty grains consisting mainly of semi-crystalline shells and an empty core (Figure 4b). In Figure 4c, taken at a larger magnification, it can be seen that the outer shell, which in turn consists of thin crystalline and thin amorphous layers, is also subjected to hydrolysis by a topochemical mechanism. The outer layer is in turn composed of five to six layers of 100 to 200 nm in thickness and which, apparently, are crystalline lamellae consisting mainly of amylopectin molecules. With further advancement of the reaction zone tangentially to the outer shell, fragmentation of these thin lamellae begins, followed by their disintegration into nanoscale layers. First, these layers have strong anisotropy and are flat nanosized particles with characteristic dimensions of 100–200 nm by 3–8 μm . The size of such particles is smaller than the size of blood cells, and they are of interest as adjuvants for vaccines against salmonellosis and mycoplasmosis. The pathogens of these diseases have a size of 0.7–7.0 μm and, as experts believe, will be effectively absorbed on the surface of flat starch particles, and used for vaccination of animals and birds. With longer hydrolysis and ultrasonic processing, sub-micron plates of starch particles are destroyed and more isometric nanoparticles with a characteristic size of 20–200 nm are formed.

4. Conclusions

This work proposes a chemical-mechanical method for producing potato starch nanoparticles with controlled particle size. The study of the dynamics of hydrolysis of starch grains by electron scanning microscopy and laser granulometry made it possible to propose a topochemical mechanism of staged solid-phase hydrolysis, first to nanoscale plates with a characteristic size of 0.2–7.0 μm , and then to more isometric nanoparticles with a characteristic size of 20–200 nm. This method enables obtaining adjuvants consisting of nanoparticles with a predetermined size.

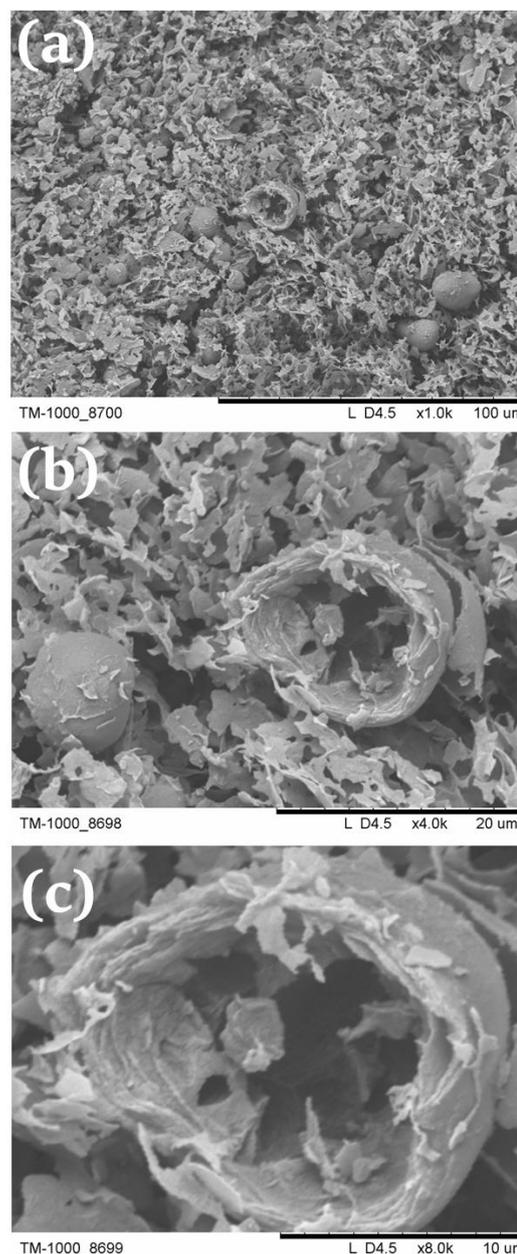


Figure 4 Starch granules after 6 days of hydrolysis at different magnifications (a, b, c).

Supplementary materials

No supplementary materials are available.

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Author contributions

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Conflict of interest

The authors declare no conflict of interest.

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