Micro and Nanostructure of Starch Granules from Potato and Maize

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The present work provides new insights about the micro and nanostructure of native starch granules from potato and maize by scanning electron microscopy (SEM) and atomic force microscopy (AFM). SEM images revealed the shape and the size distribution of the granules as well as some features, such as holes and wrinkles, on the surface of granules. These data were confirmed by AFM observations which showed the presence of numerous protrusions (nodules) on the granules surface of both starches. The structure of granules surface consisted of small spherical particles of about 30 nm in diameter, identified particularly for potato starch. These nanoparticles might be related with highly branched amylopectin molecules in substantial agreement with amylopectin blocklets (of about 20 nm) model. Larger particles of about 60 nm up to 80 nm were also visualized especially on the surface of maize starch granules representing different associations of amylopectin and amylose. The largest elongated particles of about 100 nm to 200 nm found randomly on granules of both starches might be assigned to arise from the granule-surface components, such as starch carbohydrates attached to granule proteins and phospholipids, in general agreement with starch granule surface composition data. This investigation also supports the complex structural network for the starch granule surface (periphery) and its role in maintaining the integrity of starch granules and in the starch gelatinization process.

Keywords: starch granules, potato starch, maize starch, granule surface, supramolecular structuring, scanning electron microscopy (SEM), atomic force microscopy (AFM)

Currently, there is a growing interest in the development of biodegradable plastics obtained from natural resources due to the urgent need for the reduction of plastic waste in the environment. Potato and maize starches represent the potential primary source for the production of biodegradable plastics, which are environmentally friendly and are expected to substitute the plastics made from petrochemicals. In view of complexity of the production process, a question arises related to the level of structural understanding of starch granules needed in order to engineer better plastics by design rather than trails and errors. Evidently, experimental models can be built of varying complexity depending on the available detailed description of native starches, as a first step for thermoplastic polymers production. During the fabrication process, the starches are gelatinized at different compositions in starch, water and glycerol. In order to control the gelatinization process, it is of interest to know the structure of starch granules surface.

Starch is made of polysaccharides, consisting of D-glucose units, linked together into two different macromolecules, namely amylose and amylopectin [1]. Amylose is almost linear chain based on α-1,4 linked glucose residues and sparsely branched with α-1,6 linkages. Its chain configuration is that of single helices in the granule. Amylopectin is more highly branched than amylose and it is based on both α-1,4 and α-1,6 linkages, the latter giving the branch points of the chain, at every 20-25 glucose units [2-4]. Amylopectin is present in crystalline or partially crystalline structure of the starch granule, amylose being rather amorphous [5].

The amylose/amylopectin ratio in starch granules varies according to the source, the starch from most cereals containing about 20-30% amylose, but there are starches with up to 98% amylopectin, and also high amylose starch with 60-80% amylose [6]. The starch granules from different plants have different dimensions between 0.5 and 175 μm and various shapes, such as spherical, oval, disk, polygonal, rods [7, 8]. In the starch granule, amylose and amylopectin molecules seem to be structured in growth rings [5], while at the periphery of the starch granule, a tightly associated amylose and amylopectin network is formed [9, 10]. The size, shape and surface morphology of starch granules are therefore important topics to be known for the different practical applications of starches.

Among imaging techniques, scanning electron microscopy (SEM) proved itself to be a valuable method for the determination of microstructure and surface characteristics of starch granules [1, 11-20]. This technique allows for observations of sample characteristics at nanometric resolution.

On the other hand, the atomic force microscopy (AFM) provides an ideal tool for probing starch granule structure at the molecular level. AFM studies have been performed on starch granules for starches from different sources [14, 17, 18, 21-32]. However, the ultrastructure of starch granules is not completely understood.

The advantages of the AFM over SEM are the minimal sample preparation, the ability to image under ambient conditions, for instance in air, under almost native structural conditions. The image contrast is obtained by coupling the topography, phase image and amplitude (errors image). In addition, atomic force microscopy (AFM) can go to molecular or even atomic resolution (1-2Å vertical resolution and less than 1 nm lateral resolution).

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The goal of the present work is to reveal by SEM and AFM the micro and nanostructure of starch granule surface from native maize and potato starches, which we intend to use for biodegradable plastics production.

Experimental part

Two commercially available starches, namely native potato and maize starches from Romanian cultivar were purchased from a Cluj-Napoca, Romania Company. The humidity of the starch samples was about 12%, by the manufacturer analysis.

Scanning electron microscopy (SEM)

The size, shape and surface features of the starch granules were investigated by SEM with the secondary electron imaging method (SEI). The sample for SEM imaging was prepared as follows; a small amount of starch powder was sprinkled onto a piece of conductive (carbon-impregnated) double sided adhesive tape, which was affixed to an aluminium stump. The excess of starch powder was removed by gently blowing, before gold-coating, using the AGAR, Auto Sputter Coater. Then, a thin gold coating (thickness 10 nm) was sputtered in 3 cycles taking about 10 s each on each SEM sample. Each SEM sample was viewed using a JSM 5510 LV at accelerating voltage of 10 kV and at 10.3 to 10.7 mm sample distance and with a spot size of 3 to 3.5 μm, at various magnifications from 100 to 10,000 times.

Atomic Force Microscopy (AFM)

AFM images were recorded using a AFM-JEOL 4210 operated in tapping mode, thus allowing for the simultaneously topography, phase and amplitude images for each starch sample. Granules of native starches were spread out on a double adhesive band which was affixed to AFM sample support. For the sake of comparison, the starch granules were also scattered on a freshly cleaved Muscovite mica surface, where through electrostatic interactions the granules are holding in place as a thin film. Samples were imaged in air with a scanner (25 μm x 25 μm maximum scan size) under normal air conditions of room temperature (about 22°C) and at atmospheric pressure. All images were recorded in tapping mode using commercially available sharpened silicon nitride (Si_3N_4) probes. The conical shaped tips were on cantilevers with a resonant frequency in the range of 200 - 300 kHz and with a spring constant of 17.5 N/m. The photodiode response was calibrated and the optical laser alignment was constant throughout each experiment.

Both a low scanning rate, 1 Hz, and a higher rate, in the range 2-6 Hz were used, in order to detect possible scanning artefacts or those resulting from the sample preparation. The scanning angle was also modified on different directions, in order to distinguish between real images and those corresponding to artefacts. The AFM images consist of multiple scans displaced laterally from each other in y direction with 256 x 256 pixels. All AFM experiments were carried out under ambient laboratory temperature conditions as previously reported [33, 34].

AFM observations were repeated on different areas on the scanned surface (i.e. for different magnifications), resulting in scanned areas from 20 μm x 20 μm to 1 μm x 1 μm or scaled down even more (0.5 μm x 0.5 μm) for the same sample. The AFM images were obtained from at least six macroscopic zones separately identified on each sample. All the images were processed according to standard AFM proceeding, as described for example in [35-37].

In particular, on each thin film of starch granules, AFM images were recorded at least at six macroscopically different locations on the surface, with each of the locations separated by at least 2 or 3 μm. All imaging data were analyzed using JEOL standard software.

Results and discussion

Size, shape and surface morphology revealed by SEM imaging

A selection of SEM (SEI imaging method) micrographs of starch granules from potato spread out in the thin film are given in figure 1 for two different magnifications, in order to display the surface features of granules. The analogous micrographs of starch granules from maize are given in figure 2. All granules are well defined with sharp granule contours (figs. 1 and 2) for both native starches.

As seen in figures 1, 2, the granules present a variety of appearances, such as regular shapes from spherical or elliptical and oval to irregular polygonal forms with rather smooth surfaces (figs. 1b and 2b), whereas for other granules the surface features are rather pronounced (figs. 1a and 2a). However, both types of starches contained spherical granules as can be observed in figures 1b and 2b.

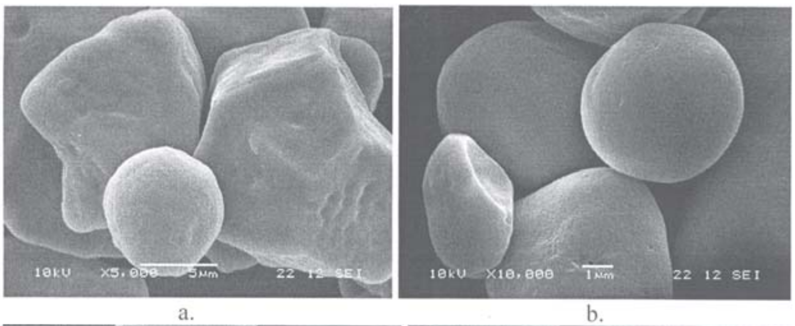

Fig. 1. SEM micrographs of potato starch granules; the magnification and the length of bar are respectively: (a) x5000; 5 μm; (b) x 10000; 1μm

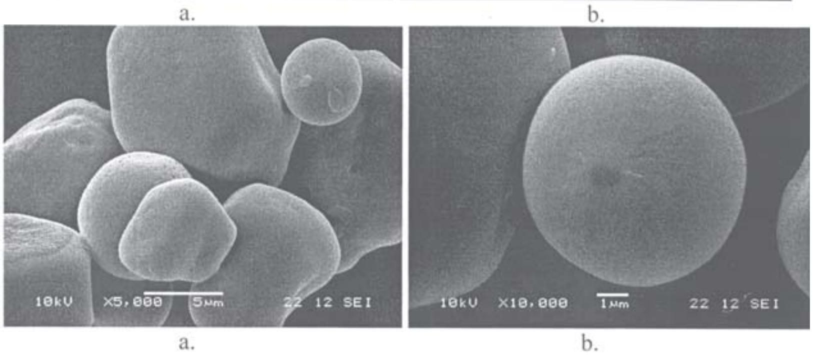

Fig. 2. SEM micrographs of maize starch granules; the magnification and the length of bar are respectively: (a) x5000; 5 μm; (b) x 10000; 1μm

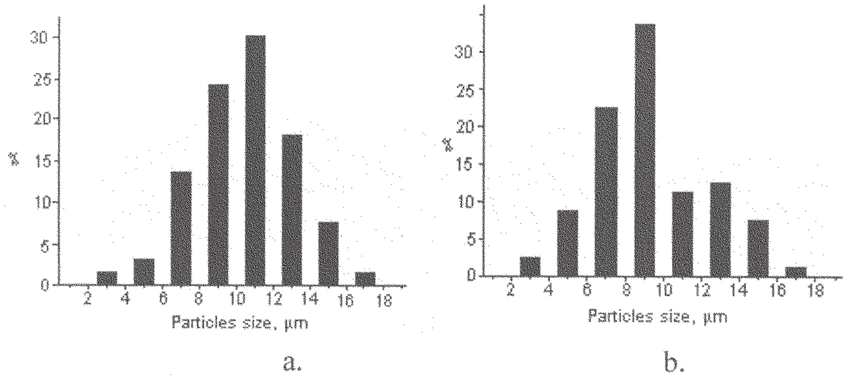

Fig. 3. Histograms of size distribution of granules in the potato starch powder (a) and the maize starch powder (b) from SEM micrographs

Referring to the surface morphology, some narrow pores or large depressions and wrinkles were observed at micro structure level on some parts of the surface of granules mainly from potato starch (fig. 1a). Occasionally, some small depressions were also seen on the granule surface from maize starch (fig. 2a). These results suggest that a structural divergence exists not only between potato and maize starches, but also on the same granule surface, in good agreement with recently reported data on a different potato starch [38].

Granule size distribution of potato and maize starches

The size distribution (fig. 3) and the average size (table 1) of granules for the two starches from potato and maize were determined by statistical analysis. The size distribution was expressed in terms of the size of equivalent spheres.

From the size of a great number of granules (several hundreds), measured on the SEM micrographs, the average size (equivalent diameter) and the standard deviation (SDEV) were calculated and are given in table 1, together with the extreme values of the granules size. It can be seen from table 1 that the size distribution is similar for the two starches and there are no significant differences between the potato and maize starch granules.

From histograms (fig. 3), it has been determined that the granule diameters between 10 and 12 µm are predominant in the potato starch powder, while the fraction of granules with diameters between 8 and 10 µm are the most numerous for the maize starch.

The fine structure of granule surface revealed by AFM imaging

AFM images of the starch granule surface are obtained, in tapping mode of AFM operation, as two dimensional (2D) and three dimensional (3D) topographies, as amplitude (errors signal) images and phase images. The contrast in AFM phase imaging facilitates the detection of variations in physical properties (such as, composition, stiffness, elasticity) of the granule surface (periphery).

Representative AFM images for two different scanned areas are given in figures 4 and 5 for native potato starch, and in figures 6 and 7 for native maize starch.

Table 1
GRANULE SIZE DETERMINED FROM SEM MICROGRAPHS FOR POTATO AND MAIZE STARCHES

Sample	Average size of granules [µm]	SDEV [µm]	Extreme values [µm]
1. Potato starch	10.3	2.7	3.8 ... 16.2
2. Maize starch	9.3	2.9	3,7 ... 16.0

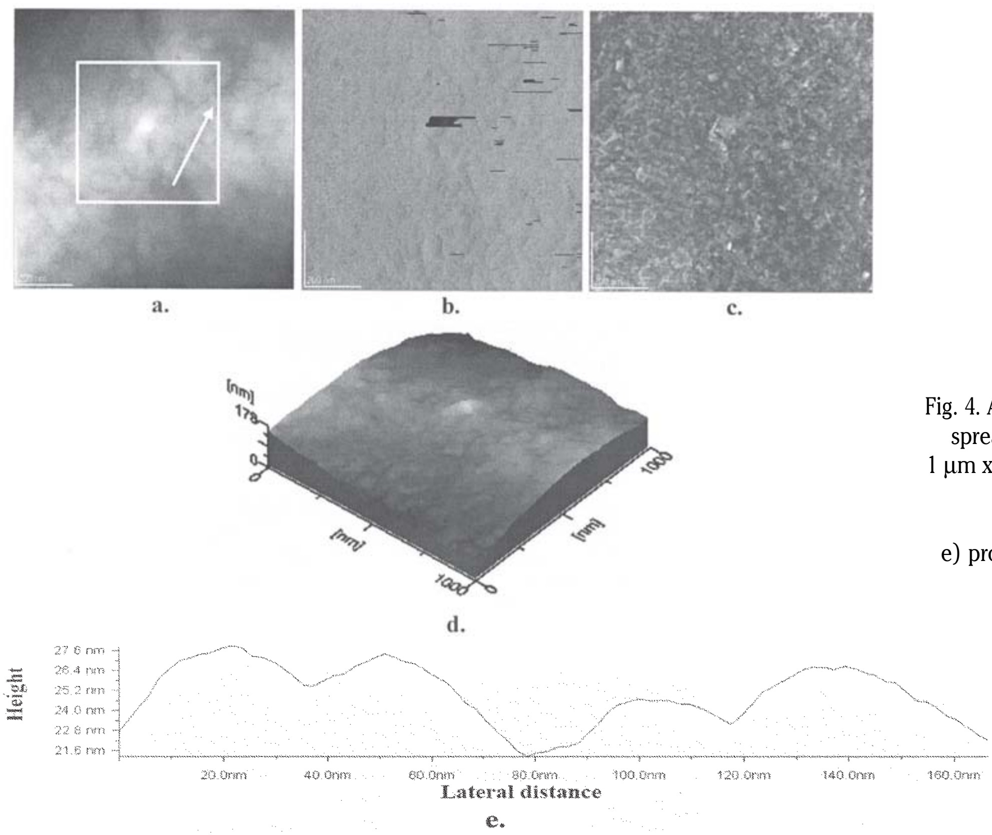

Fig. 4. AFM images of potato starch powder spread out in thin film. Scanned area: 1 µm x 1 µm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow given in panel **a**

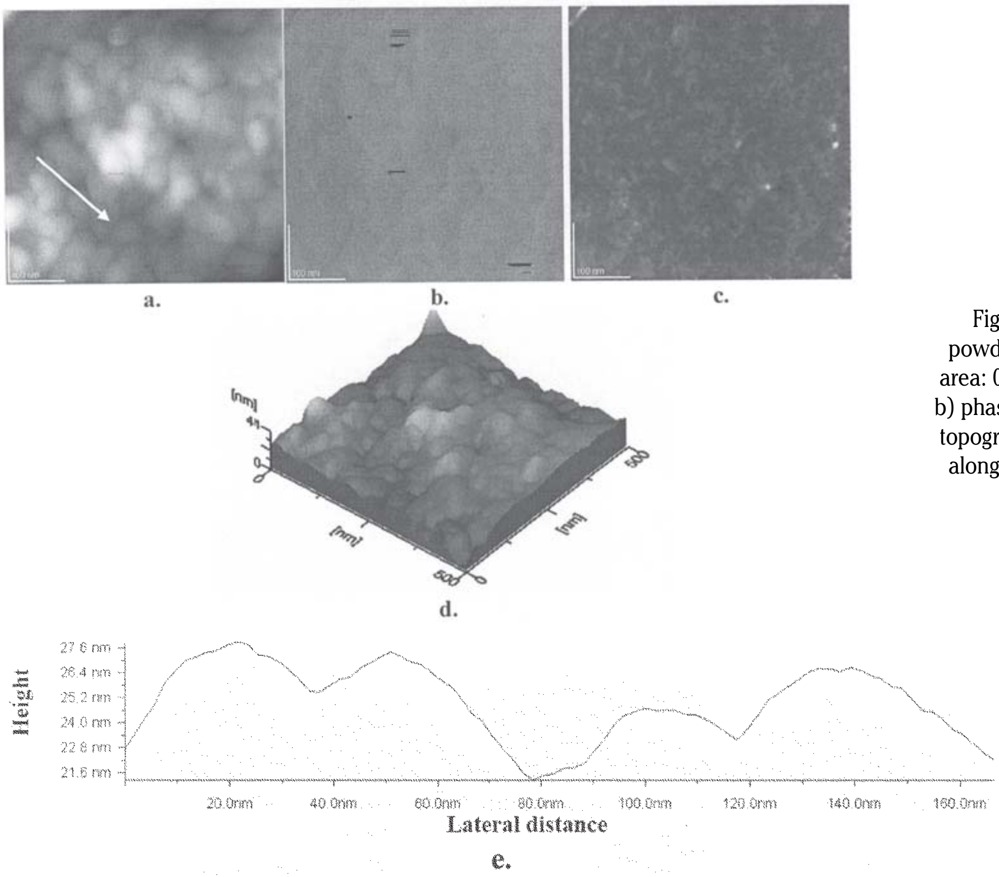

Fig. 5. AFM images of potato starch powder spread out in thin film. Scanned area: 0.5 μm x 0.5 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel a. (See marked area in fig. 4)

By comparing the AFM images in figures 4 and 5 with the corresponding ones in figures 6 and 7 a certain morphological resemblance is still observed for the surfaces of potato starch and maize starch. This situation could reflect a strong interaction between the starch macromolecules, resulting in similar particle shapes at granule surface (periphery) independent of the botanical source of starches.

From the AFM images, 2D topographies (fig. 4a-7a), and 3D topographies (fig. 4d - 7d), as well as phase images (fig. 4b -7b) and amplitude images (fig. 4c-7c), one can observe the surface structuration of the starch granules, primarily the presence of surface protrusions (small rounded nodules or particles), evidenced at small scanning areas, as given in figures 4 and 5 for the potato starch and in figures 6 and 7 for the maize starch. In the profile of the cross sections (figs. 4e - 7e) one can see the local nanostructure of the granule surface (see, arrows in Figs. 4a-7a) with nodules (nanoparticles) which protrude from the surface, generally about 30 to 40 nm in apparent diameter (figs. 4e and 5e) for potato starch and slightly larger particles, between about 60 and 80 nm (figs. 6e and 7e) for maize starch. Clearly, the granule surface presents a nanostructuration visible in AFM imaging mainly at high magnifications, respectively at small scanned areas.

The apparent diameter of the smallest features (about 30 nm) was comparable to the radius of curvature of the AFM probe tip (15-20 nm), so these images were expected to be subject to tip convolution effects.

Anyway, the observed nanoparticles, named also protrusions, subparticles or nodules, are surface features and generally present rather round or elongated shapes on the granule surface of native potato and maize starches. In addition, it is to be noted that particles in roughly the same range (about 20 to 50 nm size) were reported to be formed by precipitation with ethanol from suspensions of gelatinized potato starch [31]. They were also detected at the surface of wheat or oat starch granules [31].

The observed sizes are also in substantial agreement with the fine structure of granules of different types of starches found in granule internal [23-25, 27] and surface structure [14, 18, 21, 29-31]. For instance, small particles of about 30 nm in diameter were also found in the internal granule structure of rice [23, 24], corn [25] and pea starches. On the starch granule surface of potato and wheat starches the fine particles more or less spherical of about 25 nm were also identified [14], which were observed both within and at the surface of starch granules degraded by alpha-amylase.

These nanoparticles on the granules surface could correspond to clusters built from amylopectin side chains bundled into blocklet structures [39], evidenced earlier both on the granule surface and in lamellar structures within the starch granule [40], in agreement with the proposed cluster model [14, 31]. They can be bundled further on into larger blocklets organized within the starch granule or on the surface of granule. In other words, the smallest protrusions identified in this work, composed mainly of about 30 to 40 nm size nanoparticles might represent the ends of amylopectin side-chain clusters at the granule surface [14].

Therefore, our results support the blocklets model of the starch granule structure [39-41], independent of the starch botanical resources. Taking into account the blocklets concept, it is to be observed that the amylopectin forms smaller nanoparticles on the granule surface of native potato starch than on native maize starch. Otherwise, a more complex surface network (arrangement) might be assembled on the native maize starch granules.

Undoubtedly, the AFM images of granules surface demonstrated that the potato and maize starches possess substantially different surface topographies at nanostructure level. Even more, on the granules surface, some zones are detected with a rather high roughness, and quite smooth zones with low roughness are also observed. The roughness, measured by the root mean

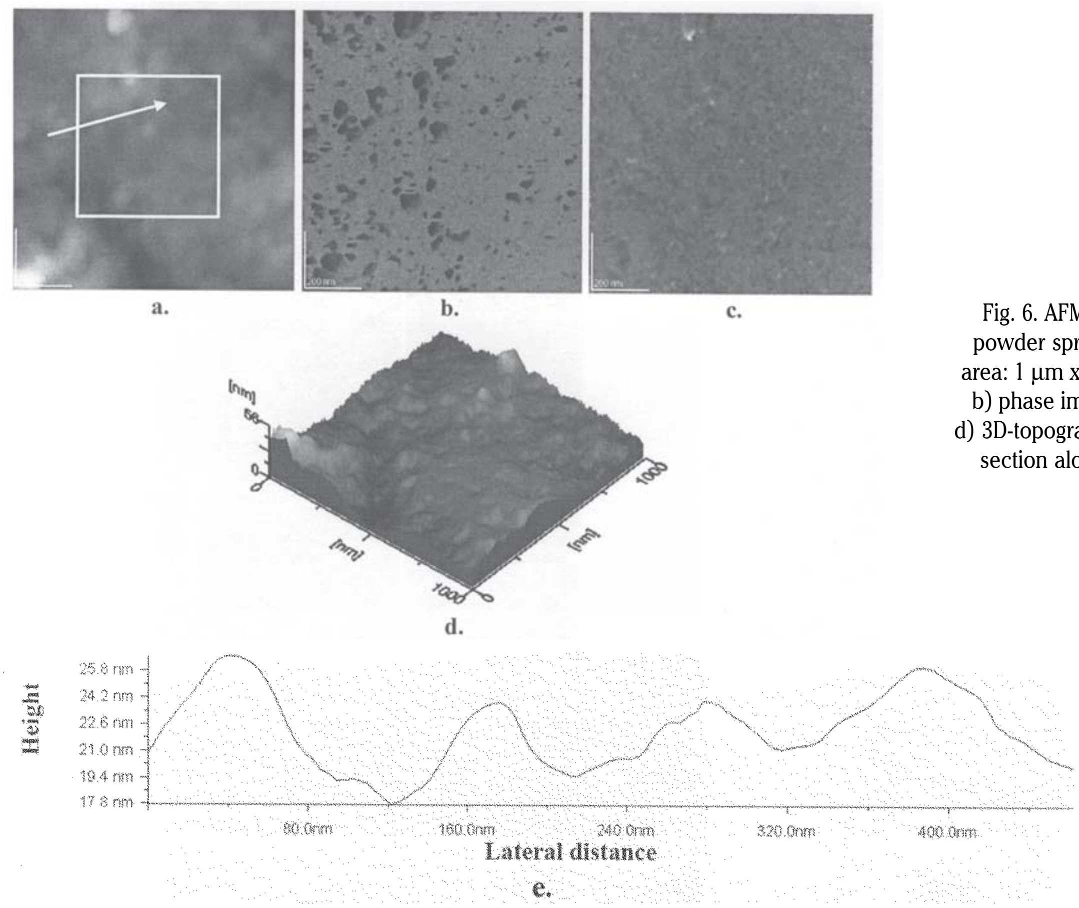

Fig. 6. AFM images of maize starch powder spread in thin film. Scanned area: 1 μm x 1 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel **a**.

square (RMS), is given in table 2 for several selected scanned surfaces. The AFM images for fairly big scanned areas, from 10 μm x 10 μm to 2 μm x 2 μm, are not shown.

Closer examination of AFM images obtained at big scanned areas (low magnification) revealed a few quite large protrusions on granules surface of potato starch of roughly 100 nm in diameter placed above the flatter surface containing the said 30 to 40 nm size nanostructures. On the other hand, the surface of maize starch granules possesses several larger protrusions of about 120 to 200 nm, and consequently an increased roughness is observed at large scanned areas (table 2). Thus, potato starch

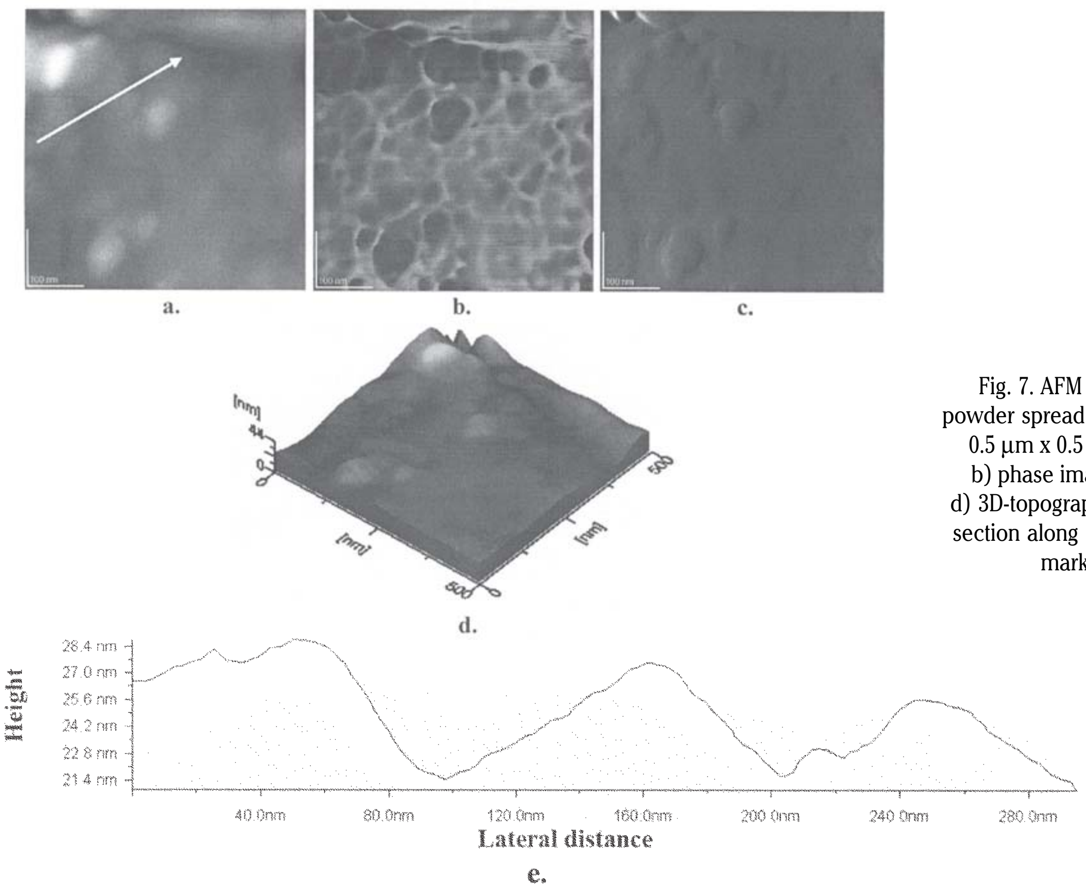

Fig. 7. AFM images of maize starch powder spread in thin film. Scanned area: 0.5 μm x 0.5 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel a. (see marked area in fig. 6)

Table 2
SURFACE ROUGHNESS GIVEN AS ROOT MEAN SQUARE (RMS) FOR THE TWO TYPES OF STARCHES

Nr. Crt.	Samples, thin films	Scanned area, μm^2	RMS on area, nm	RMS on profile, nm
1	Potato starch	10 x 10	205	206
2	- „ -	5 x 5	96	85
3	- „ -	2.5 x 2.5	67	17
4	- „ -	2 x 2	31	5
5	- „ - Fig.4	1 x 1	6	3
6	- „ - Fig.5	0.5 x 0.5	5	2
7	Maize starch	15 x 15	559	578
8	- „ -	10 x 10	525	539
9	- „ –	5 x 5	144	145
10	- „ -	2.5 x 2.5	146	14
11	- „ –	2 x 2	37	6
12	- „ - Fig. 6	1 x 1	6	3
13	- „ - Fig. 7	0.5 x 0.5	6	3

granules generally have a smoother surface (table 2) than maize starch granules. There are exceptions, for very small scanned areas when the roughness appears to be identical for both starches (table 2).

Owing to the existence of various protrusions of different size, it appears rational to suggest that the amylopectin blocklets (the smallest identified particles) are probably enlarged either by their self assemblies and/or by their attachment to other granule surface components [42-44], such as amylose, granule proteins and phospholipids. Thus, the starch carbohydrate components will possibly build a highly complex network involving the proteins or lipids attached (or bound) to the starch granule surface. Such complex surface organisation supports a highly structured surface (periphery) of starch granules and its role in maintaining the integrity of starch granule and in the starch gelatinization process.

The complex surface network might be an ordered structure, according to [45], where it is mentioned that amorphous regions are mainly located inside of starch granules, and crystalline areas mainly exist on the surface of the starch granule.

Conclusions

SEM and AFM are appropriate tools for the observation of granules surface of the two native starches, explored in the present work. SEM allows for a good visualization of the starch granules, revealing their shapes, their surfaces morphology and sizes. AFM allows for obtaining images of high resolution, without any treatment of the starch granules. The surface structures evidenced by AFM imaging, such as protruding nodules on the surface of starch granules have various sizes, in a large range of values, from 30 nm to 80 nm. Frequently, fine particles were found to self assemble on the granule surface into rather straight arrangements forming rows.

The surface organization of the starch granule is probably consisting of blocklets and the superhelix structure that have already been proposed for the association and clustering of amylopectin helices within the starch granule and on the granule surface. We suggest that the observed smallest fine particles might also correspond to the individual clusters of amylopectin in substantial agreement with the proposed cluster model and blocklets concept.

By imaging analysis of granules surface of starches, it appears that the assembly of amylose and amylopectin on the starch granule surface exhibit a highly complex organization probably involving the starch periphery attached (or bound) components, such as proteins or lipids. This complex network may give a structural support for the starch granules surface and its role in maintaining the integrity of starch granules and consequently in the starch gelatinization process.

From our investigation it appears that both, native potato and maize starches, present similar granules surface characteristics. Knowing the micro and nanostructure of starch granules it is necessary the correct specification of the processing conditions in the production of biodegradable materials, based on thermoplastic starches.

In future investigations we intend to deepen the knowledge on nanostructure of potato and maize starches from different sources, with the aim to characterize and control starches, both native and in different processing stages, in manufacturing of thermoplastic starch products.

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