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Dedicated to the memory of Prof. dr. Ioan Silaghi-Dumitrescu marking 60 years from his birth

STRUCTURE OF STARCH GRANULES REVEALED BY ATOMIC FORCE MICROSCOPY

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ABSTRACT. Atomic force microscopy (AFM) was used to reveal the micro and nanostructure of maize starch granules from Romanian cultivar. The size, shape and surface morphology of the native maize starch granules are shown by AFM observations. Numerous structures, protrusions (particles), pores or depressions and cracks were found on the surface of maize starch granules and they have a broad range of sizes. The occurrence of small spherical protrusions might be related with the highly branched amylopectin molecules in substantial agreement with the amylopectin blocklets model. The larger particles were also visualized, representing different associations of amylopectin and amylose and other granule surface components as previously discussed. The existence of rather smooth regions with low surface roughness and rougher zones on the starch granules is confirmed.

Keywords: *native maize starch, granule surface, supramolecular structuring, atomic force microscopy (AFM)*

INTRODUCTION

Currently, starch is widely used in its native form or after various modifications for plastics and food industry [1], as well as for pharmaceutical products [2, 3] and for orthopaedic implants [4]. The numerous applications of starch intensify the studies on starch granule structure due to the large variability of starch origins [1]. It is known that a differentiation in the starch granule structure and, consequently, in starch properties is related to the starch cultivar. The potential starch source in Romania might be the native maize starch, which is available in large quantities.

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The surface morphology of granules is important for the characterization of the maize starch used as raw material for various applications, including the manufacturing of biodegradable plastics. In this respect, it is already understood that the starch processing involves many interfacial modifications and the rate of such changes is controlled mainly by the surface structure of granules. The data on the surface properties of Romanian maize starch granules is unavailable yet.

Starch granules are made of polysaccharides, consisting of D-glucose units, linked together into two different macromolecules, namely amylose and amylopectin [5]. Amylose contains an almost linear chain based on α-1,4 linked glucose residues. Its chain configuration is that of single helices. Amylopectin is a highly branched carbohydrate based on both α-1,4 and α-1,6 linkages [6-8]. Amylopectin has crystalline or amorphous structure and amylose is rather amorphous [9].

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 [10]. The starch granules from different plants have different dimensions and various shapes, such as spherical, oval, disk, polygonal or rods [11, 12]. In the starch granule, amylose and amylopectin molecules seem to be structured in growth rings [9], while at the surface of the starch granule, a tightly associated amylose and amylopectin network is formed [13, 14]. Therefore, the size, shape and surface morphology of the starch granules are important data to be known for the different practical applications of starches [15-24].

Among the imaging techniques, the atomic force microscopy (AFM) provides an important tool for probing starch granule structure at molecular level. AFM studies have been performed on starch granules for starches from different botanical sources [18, 21, 22, 25-36]. However, the ultrastructure of the starch granules is not completely understood. The advantages of the AFM technique are related to the sample preparation, the ability to image under ambient air conditions, under almost normal structural conditions for the starch granules. The images are obtained as 2D- and 3D-topographies, phase and amplitude (error signals) images. In addition, the atomic force microscopy (AFM) can go to molecular or even atomic resolution (1-2 Å vertical resolution and less than 1 nm lateral resolution) [37-41].

In the present work we provide the micro- and nanostructure data by AFM imaging, such as size, shape and surface morphology, for native maize starch powder spread out in thin films or compacted into tablets.

RESULTS AND DISCUSSION

Size, shape and surface morphology of the starch granules

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 the AFM phase imaging makes possible the detection of variations in properties (such as: phase composition, stiffness, elasticity) of the granule surface (periphery).

A selection of AFM images for maize starch granules compacted into a tablet is given in Figures 1-3.

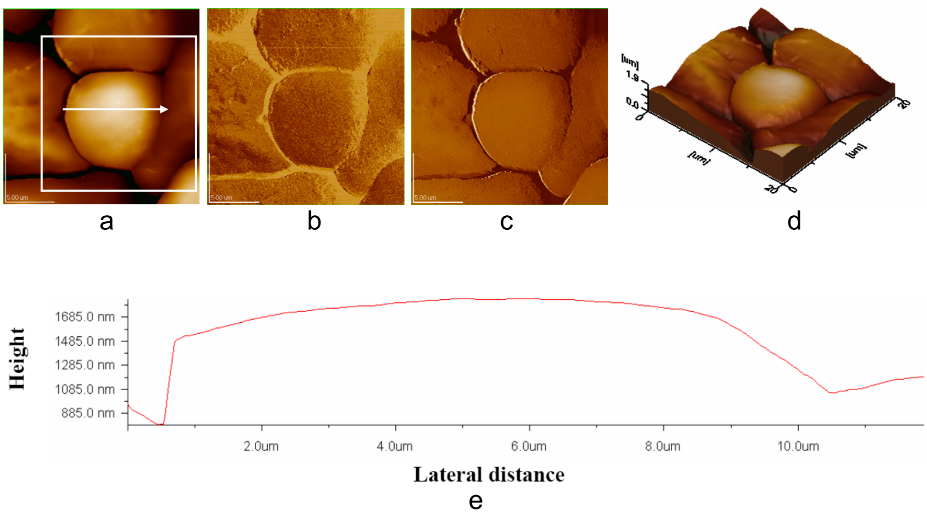

Figure 1. AFM images of maize starch compacted as a tablet. Scanned area: 20 μm x 20 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross-section along the arrow given in panel a.

Figure 1 shows a group of several starch granules with rather sharp granule contours. The granules of maize starch have mainly round and oval shapes (Figs 1 and 2). The central granule (see marked area) in Figure 1 is re-scanned in Figure 2, and it has a deformed spherical shape, with the diameter fluctuating between 9.0 μm and 9.5 μm. The height difference is between 1250 and 1300 nm (Figure 1e and 2e). Frequently, on the tablet surface, oval granules are found and their larger axis ranges from 5 to 16 μm.

By accurate imaging analysis, several depressions and pores of undefined oval shape are clearly observed in Figure 1 (a, b), particularly on the granule surface on the right of 2D-topography and phase images, and they are generally below 1 µm in size. The surface structures are detected on the profile (which is slightly undulated) of the cross-section (Figure 2e) along the arrow on the granule in Figure 2a.

Furthermore, the surface structures are clearly observed in the marked area, given in Figure 2a, at an enlargement corresponding to a re-scanning area of 1 µm x 1 µm, as shown in Figure 3.

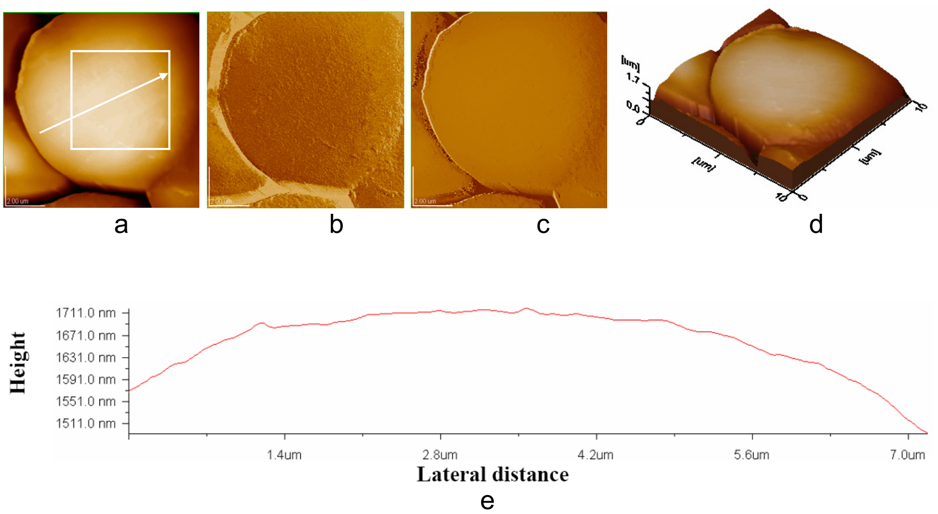

Figure 2. AFM images of compacted maize starch powder (tablet). Scanned area: 10 µm x 10 µm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel a.

Figure 4-7 display the structure of outermost layer of maize starch powder spread out in thin film for several different magnifications (selected scanning areas), in order to prove the microstructure and the surface features of starch granule surface.

Figure 4 presents an oval shaped starch granule, which shows a rather regular contour (Figure 4a-c), with the long axis of about 5.6 µm (Figure 4d and the arrow in Figure 4a) and the difference in height of about 2000 nm (Figure 4d). Figure 5 shows a starch granule with irregular contour (Figure 5a-c), although it resembles to a rather oval shape (Figure 5d) with short axis of about 6 µm, estimated from the cross-section profile (Figure 5e) along the arrow given in Figure 5a. Its long axis, determined from Figure 5a, is about 8 µm. On the 3D-topography (Figure 5d) the structure of the granule surface is rather visible and it is significantly enhanced at higher magnifications, respectively at smaller scanned areas.

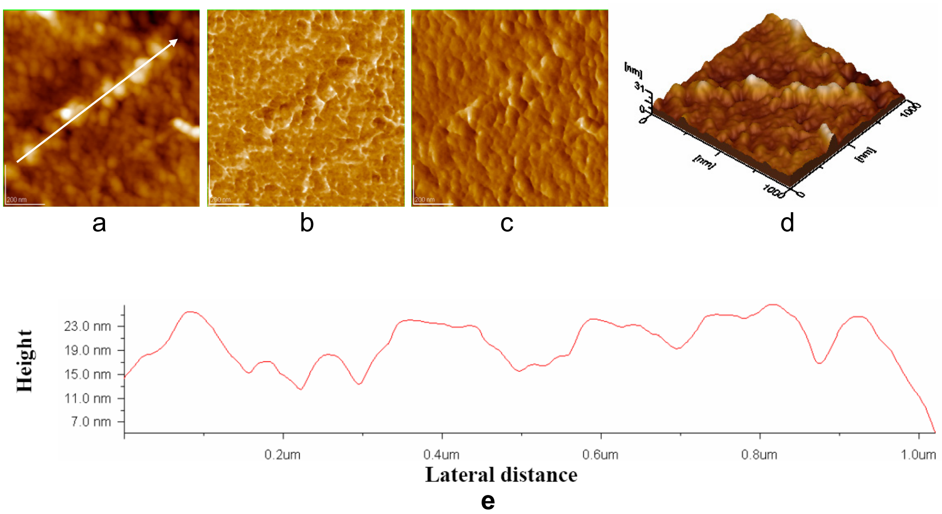

Figure 3. AFM images of the compacted maize starch powder (tablet). Scanned area: 1 µm x 1 µm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel a.

For example, Figure 6 and 7 display clearly the nanostructure on the starch granule surface at two different areas scanned on the oval maize starch granule from Figure 4.

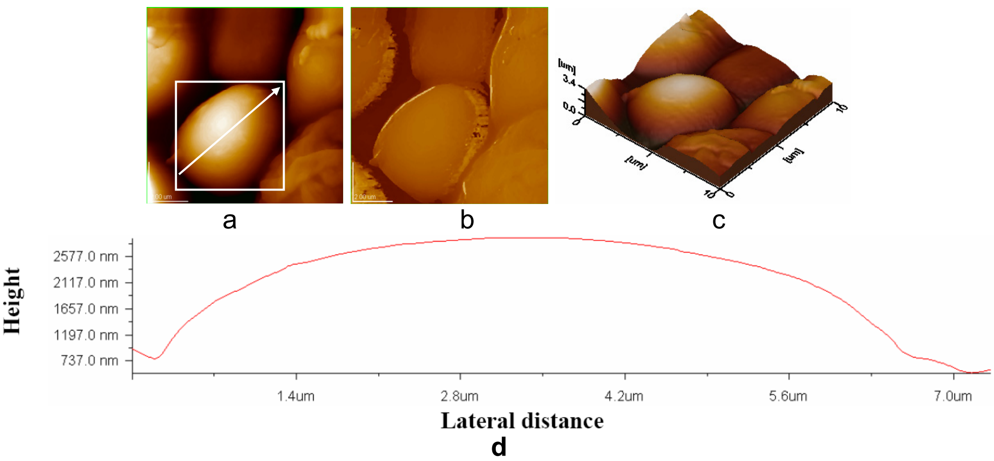

Figure 4. AFM images of the maize starch powder spread out in thin film. Scanned area: 10 µm x 10 µm. a) 2D – topography; b) amplitude image; c) 3D-topography; d) profile of the cross section along the arrow given in panel a.

From Figures 1, 2, 4 and 5, it is to be observed that almost all granules are well defined with rather sharp granule contours independently of sample preparation method. The granules of maize starch have mainly round and oval shapes, both in tablets and in thin films of powder spread out on adhesive tape. Occasionally, polygonal shapes of starch granules were also observed.

These data suggest that in the tablets obtained by compression of the maize starch powder, the starch granules are quite well packed and consequently, the granules show a rather good compactibility and compressibility. They also indicate a low surface fragility of granules surface in substantial agreement with the compaction behaviour reported on other starch samples [2, 3]. The compaction and the fragility behaviour of the starch powder is important in various applications such as in plastics production and for drug delivery systems. Undoubtedly, these observations might have implications in the formulation of poorly compactable drugs, starch powder being an important component [3] in such systems.

According to the AFM images, the granules of maize starch present a variety of forms, such as regular shapes from rather spherical (Figure 1 and 2) or elliptical and oval (Figure 4) to irregular oval form (Figure 5) or polygonal shape with rather smooth or rough surfaces with depressions or irregular holes of undefined shape. The surface roughness determined as root mean square (RMS) is given in Table 1.

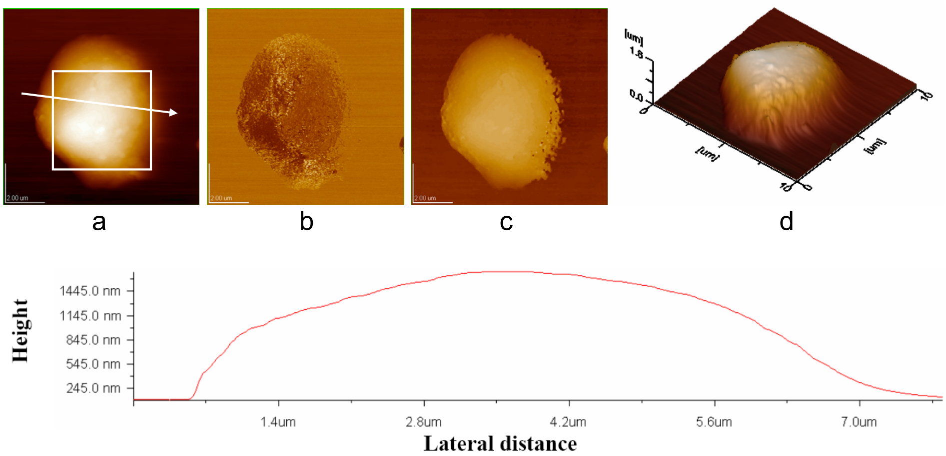

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Figure 5. AFM images of the maize starch powder spread out in thin film. Scanned area: 10 μm x 10 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross-section along the arrow in panel a.

Table 1. Surface roughness given as root mean square (RMS) for maize starch powders.

Figures	Scanned area, μm^2	RMS on tablet profile, nm	RMS on thin film profile, nm
Fig. 1	20 x 20	298	-
Fig. 2	10 x 10	55.3	-
Fig. 3	1 x 1	5.11	-
Fig. 4	10 x 10	-	568
Fig. 5	10 x 10	-	555
Fig. 6	2 x 2	-	12.4
Fig. 7	1 x 1	-	6.17

The granule size distribution is not deduced by AFM imaging, because a very large number of granules must be scanned for that purpose. The size distribution for the maize starch granules is obtained by scanning electron microscopy (SEM) measurements (unpublished results). The average size of the starch granules is about 9.3 μm with a standard deviation of 2.9 μm and with extreme values between 2 and 25 μm.

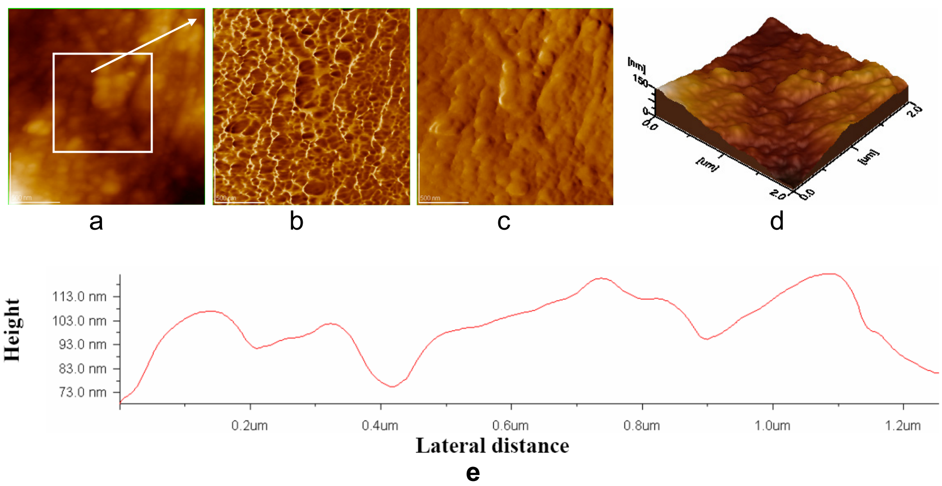

Figure 6. AFM images of maize starch powder spread as a thin film. Scanned area: 2 μm x 2 μm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross-section along the arrow in panel a.

Referring to the surface morphology, narrow pores or rather large depressions were observed at microstructural level on some parts of the surface of starch granules. These results suggest that structural differences exist even on the same granule surface, in good agreement with recently reported data on potato starch [42].

The fine structure of starch granule surface

Figure 3, 6 and 7 display numerous surface features of maize starch granules, which are rather pronounced for these magnifications.

By comparing the AFM images in Figure 3, given for the starch granules packed into a tablet, with the corresponding ones in Figure 6 and 7, for starch powder spread out in thin films, a certain morphological resemblance is clearly observed for the surfaces of maize starch granules. These data show that not only the microstructure of the starch granules is similar, but also the ultrastructure of the granule surface is comparable and practically independent of sample preparation method. This situation could reflect a strong interaction between the starch macromolecules, resulting in similar particle shapes at granule surface (periphery).

From the AFM images, 2D topographies (Figure 3a, 6a and 7a), and 3D topographies (Figure 3d, 6d and 7d), as well as phase images (Figure 3b, 6b and 7b) and amplitude images (Figure 3c, 6c and 7c), one can observe the surface structuration on the starch granules, primarily the presence of surface protrusions (small rounded and elongated nodules or particles).

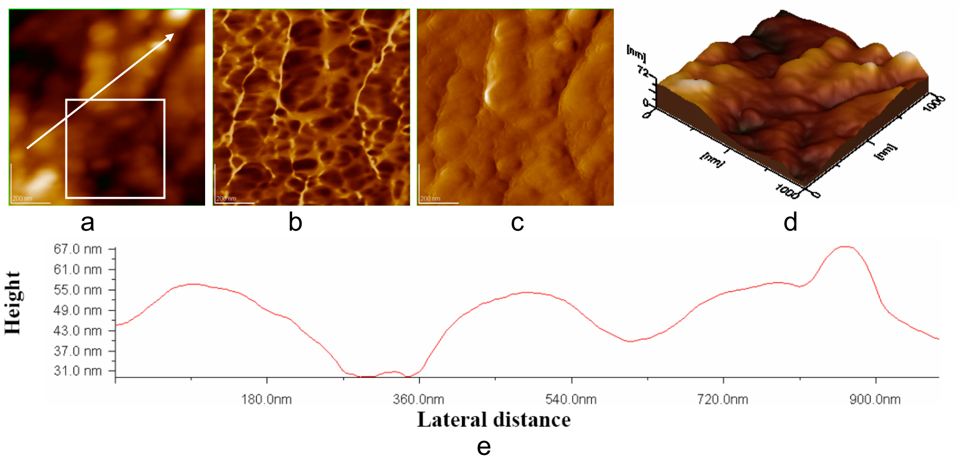

Figure 7. AFM images of the maize starch powder spread as a thin film. Scanned area: 1 µm x 1 µm. a) 2D – topography; b) phase image; c) amplitude image; d) 3D-topography; e) profile of the cross section along the arrow in panel a.

These particles are evidenced at small scanning areas, as given in Figure 3, 6 and 7 for the maize starch. In the profile of the cross-sections (Figure 3e, 6e and 7e) one can see the local nanostructure of the granule surface (see, arrows in Figure 3a, 6a and 7a) with nodules (nanoparticles) which protrude from the surface, generally between about 50 and 80 nm (Figure 3e and 7e) for the maize starch.

The apparent diameter of the smallest found features (about 30 nm) was comparable with the radius of curvature of the AFM probe tip (15-20 nm), so the images are expected to be subjected 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 the maize starch granules. 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 [35]. They were also detected at the surface of wheat or oat starch granules [35].

The observed sizes are also in substantial agreement with the fine structure of granules of different types of starches found within the granule [27-29, 31] and on the surface [18, 22, 25, 33-35]. For instance, small particles of about 30 nm in diameter were also found in the internal structure of rice [27, 28], corn [29] and pea starches granules. On the granule surface of potato and wheat starches the fine particles more or less spherical of about 25 nm were also identified [18], 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 [43], evidenced earlier both on the granule surface and in lamellar structures within the starch granule [44], in agreement with the proposed cluster model [18, 35]. They can be bundled further on into larger blocklets organized within the starch granule or on the surface of granule.

In other words, the small protrusions identified in this work, composed mainly of about 30 to 50 nm size nanoparticles might represent the ends of amylopectin side-chain clusters at the granule surface [18].

Therefore, our results support the blocklets model of the starch granule structure [43-45], independent of the starch botanical resources. According to the blocklets concept, the amylopectin forms nanoparticles on the granule surface of native maize starch. Furthermore, a complex surface network (arrangement) might be assembled among the different components bound on granule surface of the native maize starch.

Closer examination of the AFM images revealed a few quite large protrusions on granules surface of maize starch of roughly 120 nm in diameter placed above (Figure 3 and Figure 7) the flatter surface containing the 30 to 50 nm size nanostructures mentioned above.

On the other hand, the surface of the maize starch granules also possesses several larger protrusions of about 150 to 200 nm (low magnification, Figure 5).

Undoubtedly, the AFM images on the granules surface have demonstrated that the maize starch powder possesses substantially similar surface structures on different granules at nanostructural level for both tablet and thin films.

Even more, on the same granule 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 square (RMS), is given in Table 1 for several selected scanned surfaces.

The surface roughness is apparently higher for the outermost layer of the thin film than for the corresponding value for the tablet surface (Table 1). This is a somehow expected situation due to the compressibility of the granules within the tablet.

There are exceptions, for very small scanned areas, when the roughness appears to be almost identical for both maize starch tablet and thin film (Table 1).

Due 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 [46-48], 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 the existence of a highly structured surface (periphery) of starch granules according to [49] and its role in maintaining the integrity of the starch granule. The effect of starch granule surface can be also evidenced in the starch gelatinization process, where chemical modifications of the granule surface take place.

CONCLUSIONS

We can conclude that the AFM technique is an appropriate tool for the observation of granules surface of the native maize starch. AFM allows a good visualization of the starch granules, revealing their shapes, surfaces morphology and sizes. The surface structures evidenced by AFM imaging, such as protruding nodules on the surface of the 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 (Figure 3 and 7).

The surface organization of the starch granule is probably consisting of blocklets as structural elements 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.

In future investigations we intend to deepen the understanding of the nanostructure of native maize starch with the aim to characterize and control the raw starch material, both native and in different processing stages, such as in gelatinization process, in manufacturing of thermoplastic starch products.

EXPERIMENTAL SECTION

A commercially available maize starch from Romanian cultivar was purchased from Nordic Invest, Cluj-Napoca, Romania. The humidity of the starch samples was about 12%, by the manufacturer analysis. The native maize starch powder was used as supplied.

AFM images were recorded using a AFM-JEOL 4210 (JEOL Ltd. Tokyo, Japan) operated in the tapping mode, thus allowing for the simultaneously topography, phase and amplitude images for each starch sample.

Starch samples were prepared by two methods, namely the starch powder was compacted into tablets or directly spread out in thin films on an adhesive tape. The starch tablets were prepared as follows: the starch powder (around 1g) is compressed in a hydraulic press in vacuum, without any binding agent. Starch powder was also spread on a double adhesive band, on which the starch granules are holding in place as a thin film. Then, each starch sample, tablet or thin film, was independently affixed to the AFM sample support. The outermost layer of starch tablet or of the thin film of starch granules was imaged in air with a scanner (30 μm x 30 μm maximum scan size) under normal air conditions, at room temperature (about 22 ^{o}C) and at atmospheric pressure.

All images were recorded in tapping mode using commercially available sharpened silicon nitride (Si_3N_4) probes (Mikromasch, Estonia). The conical shaped tips were on cantilevers with a resonant frequency in the range of 200 - 300 kHz and with a spring constant of 48 N/m.

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 method. 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 [37, 38].

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 [39-41].

In particular, on each sample 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.

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