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SEM CHARACTERIZATION OF STARCH GRANULES

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ABSTRACT. Maize and potato starch samples were investigated by three scanning electron microscopy techniques (secondary electron imaging, Everhart Thornley secondary electron detection, backscattered electron imaging) at different magnifications. The starch granules were visualized and their shapes, surface morphology and size were revealed. From the SEM images the size distribution of the granules was inferred and compared for the different samples. These investigations will contribute to the characterization of native starches as raw materials for the production of biodegradable plastics.

Keywords: *potato starch; maize starch; SEM; granulometry*

INTRODUCTION

In the current search for “green” alternatives to petrochemical based plastic products, starch remains in the top of the options. This biopolymer presents numerous advantages, such as its abundance, low cost and biodegradability [1]. Excepting its use as filler in starch-filled polymer blends [2], in the production of biodegradable plastics, native granular starch has to be modified (destructured), giving thermoplastic starch or foamed starch [3-5]. It is also used in various blends with synthetic polymers, both non-biodegradable (such as polyethylene) and biodegradable (polylactic acid, PLA, polycaprolactone, PCL etc.).

Starch is a polysaccharide, consisting of D-glucose units, linked together into two different macromolecules: amylose and amylopectin. Amylose contains linear or sparsely branched chains based on α-1,4 glucosidic bonds and has a molecular mass of 10^5-10^6; the chains configuration is that of single or double helixes. Amylopectin, on the other hand, is based on both α-1,4 bonds and α-1,6 linkages, the latter giving the branch points of the chain, at every 20-25 glucose units The molecular mass of this multiple branched polymer [6-8] is as high as 10^7-10^9, and it is present in the semi-crystalline structure of the starch granule, amylose being amorphous [9].

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Starch is known to be the main storage substance in plants (cereals, legumes and tubers). Main sources for separation of starch granules are potato, maize, wheat, rice, pea, cassava (manioc) etc. The amylose/amylopectin ratio in starch granules varies according the resource, the starch from most cereals containing 20-30% amylose. In waxy maize (*Zea mais cerata*) and waxy rice, starch contains almost only amylopectin (98%), while high amylose maize starch and some species of peas contain 60-80% amylase [10]. Native starch granules from different plants have dimensions between 0.5 and 175 μm and various shapes: spherical, oval, disk, polygonal, rods [11, 12]. Within the starch granule, amylose and amylopectin molecules seem to be interdispersed [9], while amylose is concentrated at the periphery of the granule, where a tightly associated amylose and amylopectin network is formed [13, 14].

The surface morphology, size and shape of starch granules are therefore important topics to be known for the different practical applications of starch. Scanning electron microscopy (SEM) proved itself to be a valuable method in the study of the granulate microstructure and surface characteristics of starch and for the investigation of the enzymes effect on starch granules [15-23]. This technique allows for an observation of the sample characteristics at nanometric scale, as compared with the 0.2 μm resolution available in the optical microscope. Previously, we reported on the surface characteristics of starch granules using atomic force microscopy (AFM) investigations [24].

The goal of the present work is to reveal, by SEM observations, the size, shape and surface morphology of starch granules from native maize and potato starches, which are used for the production of biodegradable plastics.

RESULTS AND DISCUSSION

Some of the SEM images of the starch granules from potatoes in the thin film examined by the secondary electron imaging (SEI) technique are given in Figure 1 for different magnifications. The analogous pictures of starch granules from maize are given in Figure 2.

From the sizes of a great number of particles (some hundreds), measured on the SEM images, the average size (equivalent diameter of the granules) and the standard deviation (SDEV) were calculated and are given in Table 1, together with the extreme values of the granule sizes. The histograms providing the size distribution of starch granules, obtained from SEM pictures, are given in Figure 3. The size distribution is similar in the two samples, i.e. there are no significant differences between the potato (Fig. 3a) and maize (Fig. 3b) starch granules.

From these histograms, the granulometry of the starch samples was derived and given in Table 2, both for number of particles and particles weight. While in the potato starch powder the fraction with granule diameters between

10 and 12 μm are predominant both in number (Fig. 3a) and in weight (Table 2), for the maize starch the granules with diameters between 8 and 10 μm are the most numerous (Fig. 3b), but the major contribution to the weight is given by the larger particles, with diameters in the 12...14 μm range.

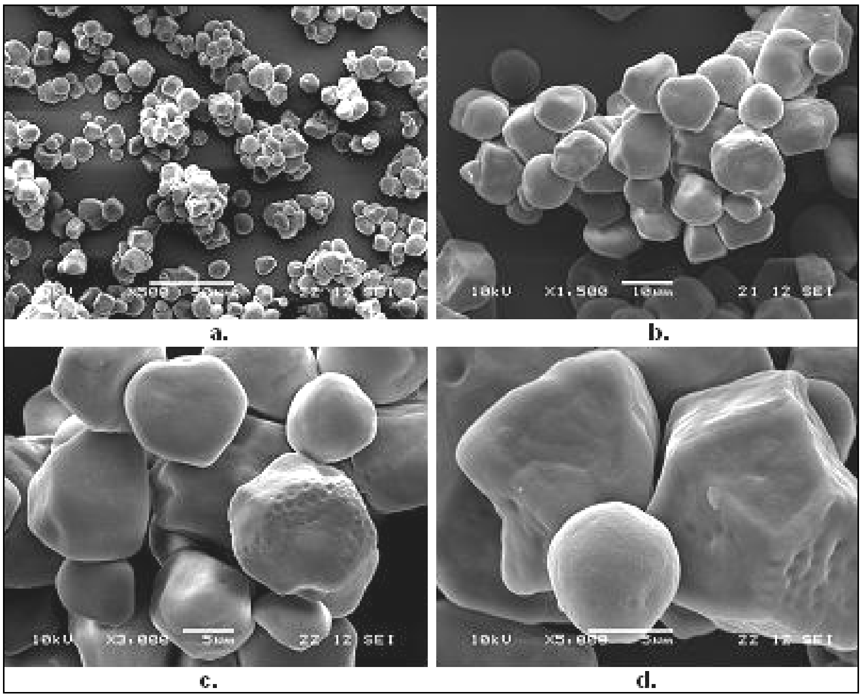


Figure 1. Potato starch granules (sample 1) visualized by SEM (SEI technique), the bar lengths are respectively: 50 μm (a); 10 μm (b); 5 μm (c); 5 μm (d).

Table 1. SEM characterization of starch granules

Sample	Average size of granules [μm]	SDEV [μm]	Extreme values [μm]
Potato starch, sample 1, thin film	10.3	2.7	3.8 ... 16.2
Maize starch, sample 2, thin film	9.3	2.9	3,7 ... 16.0
Potato starch, sample 1, compact tablet	9.5	3.5	2.2 ... 23.7
Maize starch, sample 2, compact tablet	9.3	3.3	2.2 ... 22.8
Potato starch, sample 1, from BSE	8.6	3.4	2.3 …19.1
Maize starch, sample 2, from BSE	9.2	3.9	3.2 …21.8
Maize purified starch, sample 3	11.1	6.6	2.3 …37.3

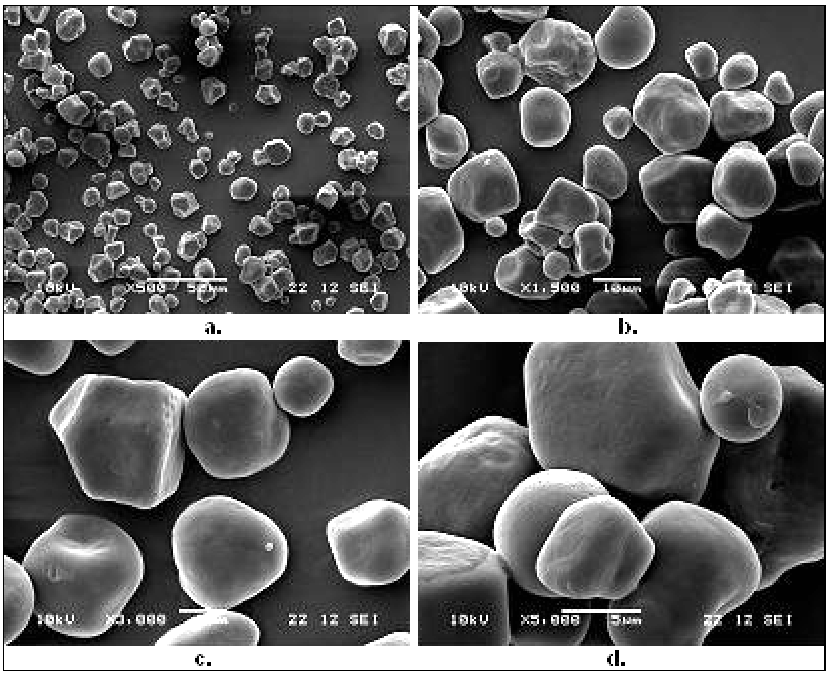
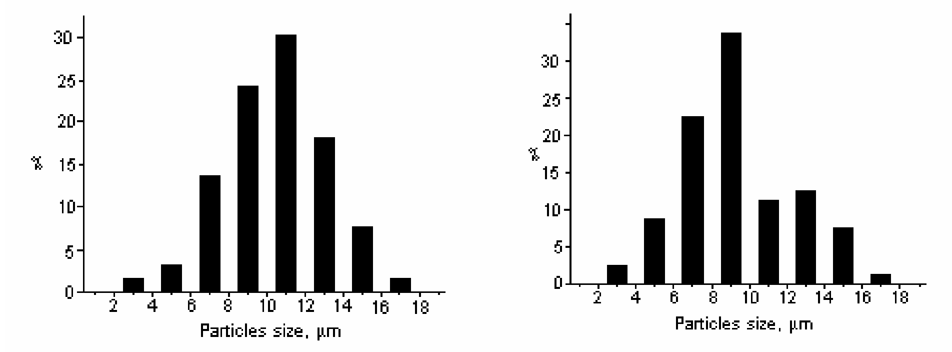


Figure 2. Maize starch granules (sample 2) visualized by SEM (SEI technique), the bar lengths are respectively: 50 μm (a); 10 μm (b); 5 μm (c); 5 μm (d).



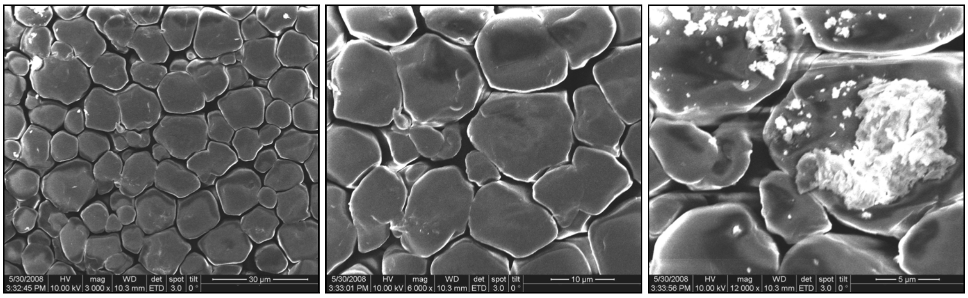
a. **b.**

Figure 3. Histograms of size distribution of granules in the potato starch sample 1 (a) and the maize starch – sample 2 (b) thin films

Some of the SEM images at different magnification ratios, obtained with the SEM-ETD technique on compact starch tablets, are given in Figure 4 for the potato starch (sample 1) and in Figure 5 for the maize starch (sample 2). The sizes of the granules, measured from the SEM images, are given in Table 1 and the histograms for the size distribution are presented in Figure 6.

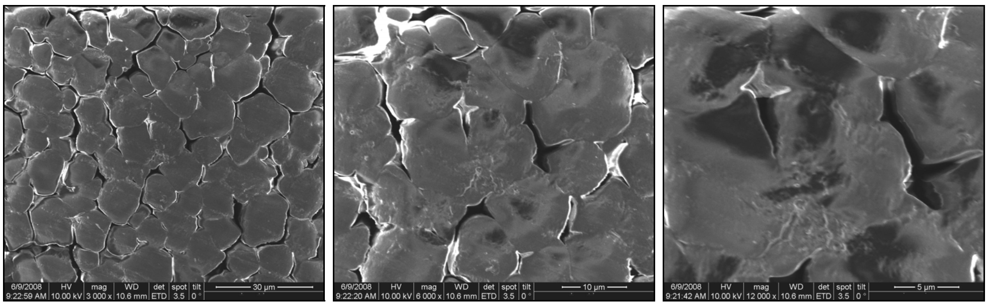
Table 2. Granulometry (size distribution) of potato and maize starch samples in thin films

Diameter (µm)	% particles number		% particles weight	
	Potato starch (sample 1)	Maize starch (sample 2)	Potato starch (sample 1)	Maize starch (sample 2)
2-4	1.8	2.4	0.0	0.1
4-6	3.2	8.6	0.3	1.0
6-8	13.6	22.4	3.4	7.1
8-10	23.9	33.6	12.8	22.6
10-12	30.1	11.3	29.4	13.8
12-14	18.1	12.6	29.2	25.5
14-16	7.7	7.6	19.1	23.6
16-18	1.6	1.4	5.8	6.3



a. b. c.

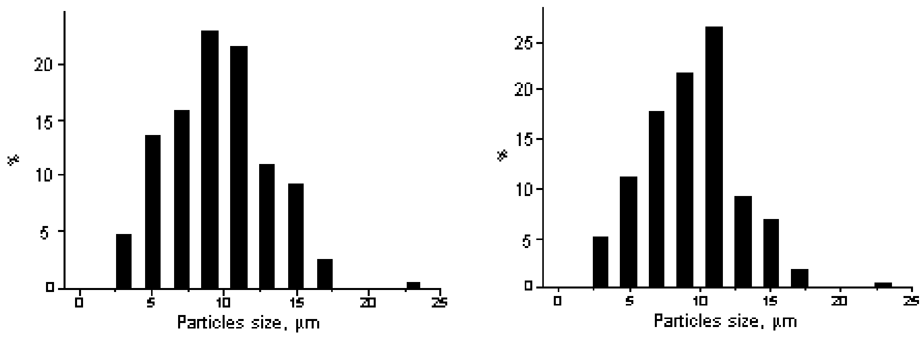
Figure 4. Potato starch granules (sample 1) visualized by SEM with ETD technique in compacted tablet; bar lengths: 30 µm (a); 10 µm (b); 5 µm. (c).



a. b. c.

Figure 5. Maize starch granules (sample 2) visualized by SEM with ETD technique in compacted tablet; bar lengths: 30 µm (a); 10 µm (b); 5 µm. (c).

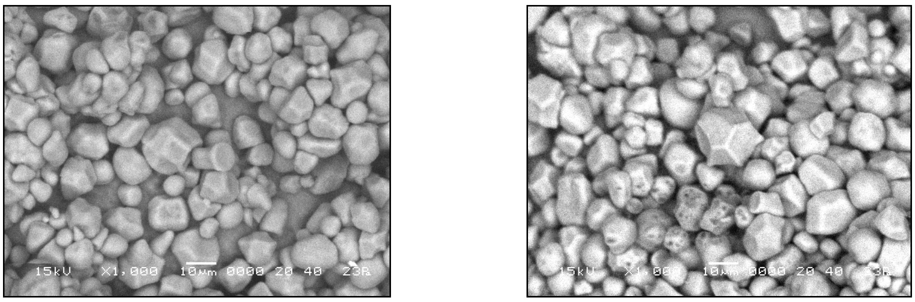
Some of the starch granules visualized in the SEM images (Figs. 1, 2, 4, 5) present smooth surfaces, for other the surface roughness is rather pronounced. A variety of particle shapes can be observed on the images, from spherical and oval to irregular polygonal shape. These shapes are more or less conserved in the compact tablets (Figs. 4, 5), but some of the particles are fragmented or present cracks (see for instance Fig. 4 c, d). These observations are consistent with the results of AFM investigations on similar starch samples [24]. AFM allows for a more detailed visualization of the ultrastructure of the granules surface. While the average size of the granules is little affected by compression, particularly for the maize starch, the distribution of the diameters is enlarged in the tablets (as seen from the standard deviation and the extreme values in Table 1). Smaller particles are present as result of the fragmentation of granules, but also larger particles result by the aggregation of granules.



a. **b.**

Figure 6. Histograms of size distribution of granules in the potato starch – sample 1 (a) and the maize starch – sample 2 (b) tablets

The SEM images obtained with the BSE technique for the same two starch samples (1 and 2) give also a clear visualization of the starch granules, as seen from the examples given in Figure 7. The size distribution is similar to that seen from the other SEM imaging techniques.

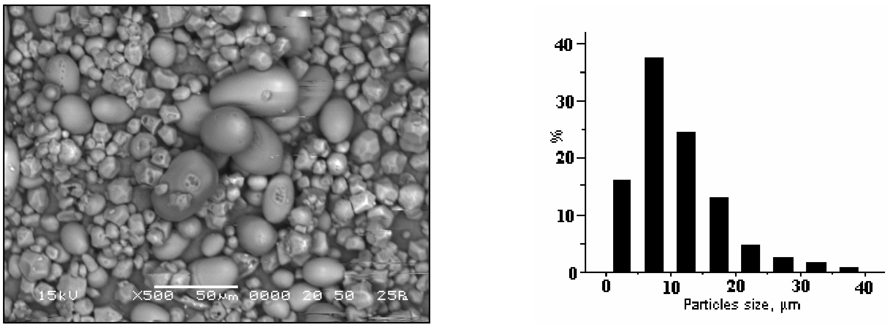


a. **b**.

Figure 7. Potato starch – sample 1 (a) and maize starch – sample 2 (b) granules visualized by SEM - BSE technique; the bar length in the images is 10 µm.

The purified maize starch (sample 3) was also investigated by the BSE technique; a typical image is given in Figure 8, along with the histogram of the particles distribution. The size distribution of the granules in particles numbers and in particles weights is given in Table 3.

As compared with sample 2, there are more small granules, but there are also a few very large granules, so that the distribution is larger (from 2 to 37 μm, standard deviation 6.6 μm) and the average size is somewhat higher (11.1 μm).



a. b.

Figure 8. Maize starch granules (sample 3) visualized by SEM - BSE technique; the bar length is 10 μm (a), and histogram of size distribution of granules (b)

Table 3. Granulometry of maize purified starch (sample 3) from SEM-BSE imaging

Diameter (μm)	% granules number	% granules weight
2-5	16.8	0.1
5-10	36.6	4.7
10-15	24.4	14.6
15-20	13.0	21.3
20-25	4.6	16.0
25-30	2.3	14.6
30-35	1.5	15.7
35-37	0.8	12.9

CONCLUSIONS

Knowing the granulate microstructure of starch is imperative for the correct specification of the processing conditions in the production process of biodegradable materials, based on thermoplastic starch.

The investigation by three SEM techniques, viz. secondary electron imaging (SEI) technique on gold sputtered samples, the use of the Everhart Thornley secondary electron detector (ETD) on starch tablets and backscattered-electron (BSE) imaging technique has enabled us to characterize commercial native starch samples from two different sources, potato and maize, as well as purified maize starch. SEM allows for a good visualization of the starch granules, revealing their shapes, their surfaces morphology and sizes. Thus, some Romanian starches were investigated for the first time and their granulometry was established from histograms based on the measurement of granules sizes by SEM images. We could also follow the effect of compression on the morphology of starch granules.

The present SEM studies complete our AFM observations on the same type of starches, which helped to better understanding of the ultrastructure of the granule surfaces. These techniques will be applied in our laboratories to characterize the thermoplastic starch products obtained from the investigated native starch samples.

EXPERIMENTAL SECTION

The investigations were made on commercial native potato (sample 1) and maize (sample 2) starches, and on purified maize starch (sample 3). The moisture of the starch samples was about 12%.

The granulate microstructure of the samples was analyzed by scanning electron microscopy (SEM), with three imaging techniques, namely scanning electron microscope, JEOL JSM 5510 LV, Japan, using the secondary electron imaging technique (SEI); scanning electron microscope, FEI Company, Netherlands, with the Everhart Thornley Seconday Electron Detector (ETD) [25]; scanning electron microscope, JEOL JSM 5600 LV, Japan, using the backscattered-electron (BSE) imaging technique.

For the SEI imaging, the starch powder was deposited in thin layer on an adhesive metallic support and then was gold sputtered in the AGAR, Auto Sputter Coater. The thin gold coating (thickness 10 nm) was sputtered in 3 sputtering cycles taking about 10 s each. These metallized samples were examined on the SEM, with accelerating voltage of 10 kV, working distance between 10.3 and 10.7 mm and a spot size of 3 to 3.5 µm, at magnification ratios from 100 to 10,000 times.

With the Everhart Thornley Secondary Electron Detector (ETD), compact starch tablets can be used, without metallization. The starch tablets were prepared as follows: the starch powder (around 1g) is crushed in a hydraulic press in vacuum, without any binding agent. The accelerating voltage was also 10 kV, the working distance 21-22 mm and the spot size 12 µm. Images were obtained with magnification ratios between 800 and 12,000 times.

For the BSE imaging, the samples were deposited as an uniform layer on the adhesive graphitized tape and were examined in low vacuum (20 – 25 Pa), with an accelerating voltage of 15 kV, a working distance of 20 mm, an electron spot size of 40 or 50 µm, at magnification ratios from 500 to 1,000 times.

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