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SURFACE OF TRITICALE STARCH GRANULES - NC-AFM OBSERVATIONS

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ABSTRACT

NC-AFM (non contact atomic force microscopy) was used to observe the surface of triticale starch granules. The method allows to obtain micro-images of high resolution with no sample pretreatment. Surface structures and protrusions or pores found on the surface of triticale starch granules had a broad range of diameters. The existence of flat, smooth regions on the starch granules, with no visible structures, pores or protrusions, was confirmed.

Key words: triticale starch, granule surface, atomic force microscopy.

INTRODUCTION

Starch - one of the most common natural polysaccharides is widely used in food industry and finds many other commercial applications. It can be utilised in native form or after various modifications. Such a widespread use of starch and its preparations is due to its structural features and variability of starch types [19]. In the last 25 years global starch production increased about 4.5 times [19]. The growing demands for new starch products make it necessary to intensify the studies on their structure, properties, modifications and applications. According to Röper [23] the potential starch sources in Europe could be barley, buckwheat, oat, rye and triticale.

Triticale exhibits very high yield, beneficial chemical composition and high resistance to mildew and rust. Its soil requirements are lower than for wheat, so triticale could compete with other cereals, especially on medium soils. Flour obtained from triticale grain could be useful in manufacturing of bread and pastry [11]. According to Fortuna et al. [8] triticale grain contains high amount of starch (73 - 85%), which has no unwanted features in comparison to wheat starch.

The granules of triticale starch have bimodal size distribution and can have round or oval shape. In case of oval granules their larger axis ranges from 22 to 36 micrometers [15]. The granules under 5 micrometers in diameter are considered "small". Pasting characteristics of triticale starch has shape typical for cereal starches [12, 21]. The drawback of triticale starch is its unstability related to high amylolytic activity of triticale grain.

The morphology and topography of surface is an important feature of solids used as raw material or ready to eat product [1]. Starch processing involves many interfacial changes, the rate and efficiency of them is controlled mainly by surface structure of starch granules. The information on the surface characteristics of triticale starch granules is yet unavailable, so the aim of this study was to provide such data. The observation of surfacial structure was conducted by means of atomic force microscopy, using its non-contact method (NC-AFM).

MATERIAL

Triticale starch obtained by laboratory method from "Bolero" cultivar was used as a material. The isolation method involving the following steps: 1) dough preparation, 2) dough tempering (30 °C, 60 min), 3) elution of starch using 0.1% aqueous NaCl solution, 4) centrifugation of starch suspension (1700 x g, 5 min), 5) elimination of supernatant and impurities, 6) dissolution of starch sediment, 7) repetition of steps 4 – 6 to obtain a sediment of pure starch, 8) starch drying (ambient conditions), 9) starch lumps disintegration, 10) starch sieving (mesh size 0.09 mm).

METHODS

Atomic force microscopy in the non-contact mode was performed by means of Park Scientific Instruments Autoprobe CP model (USA). The scanning system, consisting of a piezoelectric tube with a maximum XY scan range of 100 x 100 micrometers and a Z range of 7.5 micrometers was used. Vibrational isolation of the microscope was achieved by a dumping system consisting of heavy table hanged on a set of four buffer springs. The AFM measurements were performed at ambient conditions for starch sample spread directly onto a sticking tape fixed on an AFM sample holder, which was enough to immobilise the granules and prevented contamination of starch surface. All images were obtained with commercially available cantilevers with nominal spring constant of 2.1 N/m (Park Scientific Instruments). They were collected with ultralevers oscillated with the constant amplitude (in the range of 20-30 nm) and the frequency of about 0.3 kHz above the resonant frequency. The resonant frequency was 70 kHz, the scan frequency - 1 Hz.

RESULTS AND DISCUSSION

Figures 1 - 4 represent images of typical triticale starch granules. Figure 1 shows oval granules 5 x 2 micrometers in size and 3 x 1.5 micrometers in size. On the surface of the granules there could be observed some small surface structures and pores. Figure 2 displays a granule about 6 x 4 micrometers in size. The shape of the granule is somewhat irregular, although it resembles a sphere, and its surface seems to be relatively smooth. Figure 3 represents a group of three granules with comparatively regular spherical shapes. Their sizes range from about 1.5 to 5 micrometers. The granules have almost smooth surface, but small pores and holes are still present. Figure 4 displays a triticale starch granule of regular oval shape and dimensions of about 10 x 9 micrometers. Modest furrows and surface structures which are clearly visible do not change the general smoothness of its surface. Shallow depressions which also can be observed there are below 1 micrometers in size. Such a depression can be seen at a higher magnification on Figure 5. It has an irregular shape and is almost 1 micrometers in size. The furrows and surface structures are significantly enhanced at this enlargement. Figure 6 demonstrates a fragment of triticale starch granule, where apart from numerous minor surface structures, there exist some pores of undefined oval shape. The diameter of these pores is about 50 nm.

Fig. 1. NC-AFM image of triticale starch granules (scan size 5 x 5 µm, height difference 0.37 µm)

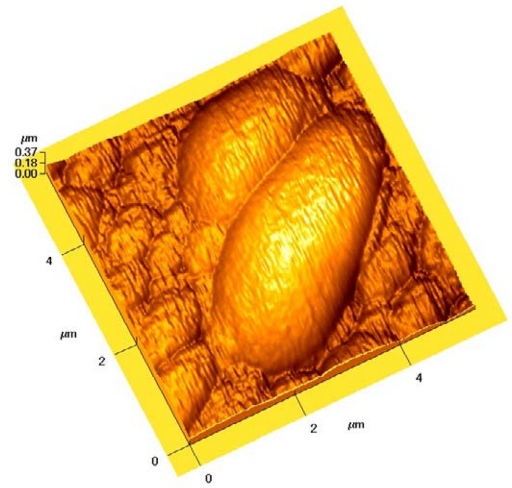


Fig. 2. NC-AFM image of triticale starch granule (scan size 8 x 8 µm, height difference 0.62 µm)

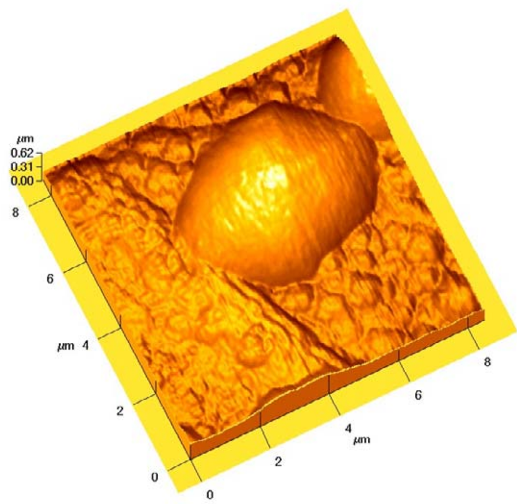


Fig. 3. NC-AFM image of triticale starch granules (scan size 9 x 9 µm, height difference 0.60 µm)

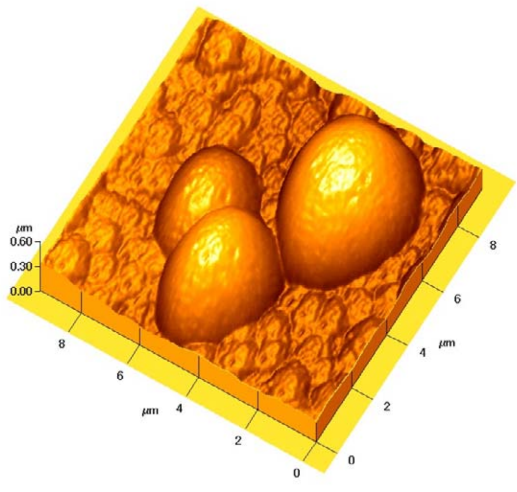


Fig. 4. NC-AFM image of triticale starch granule (scan size 12 x 12 µm, height difference 5.48 µm)

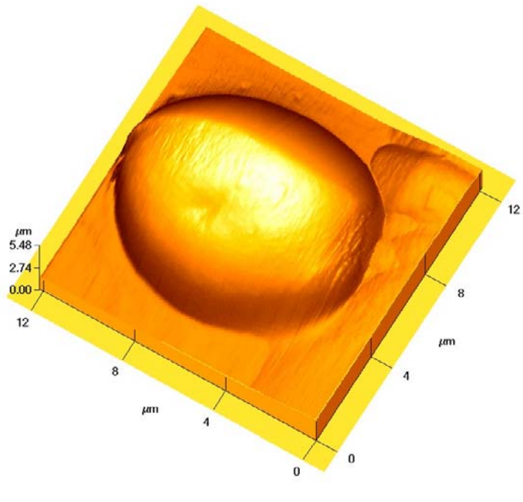


Fig. 5. NC-AFM image of surface triticale starch granule (scan size 3 x 3 µm, height difference 0.42 µm)

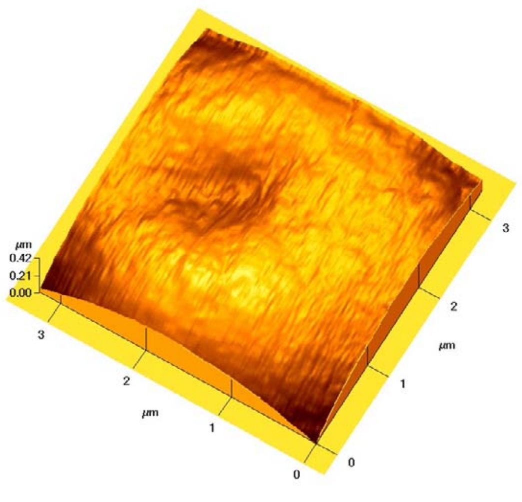
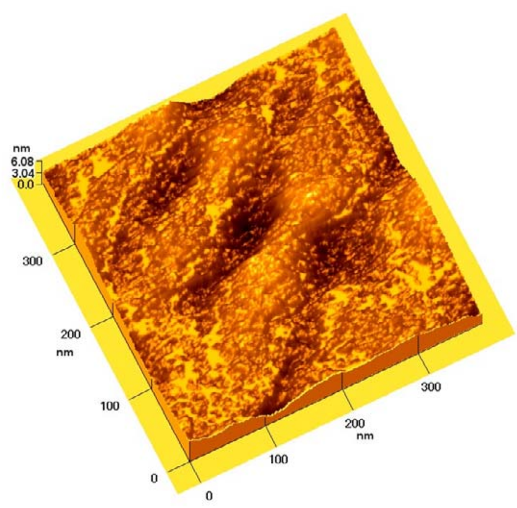


Fig. 6. NC-AFM image of surface triticale starch granule (scan size 400 x 400 nm, height difference 6.08 nm)



Earlier studies on starch granules of different origin revealed the presence of pores and minor surface structures [3, 4, 5, 6, 7, 16, 17, 20]. Whistler et al. [26] observed that the number of pores or fractures found on the surface of starch granules depends on moisture content and drying method. These authors also stated, that such pores facilitate absorption of chemical reagents. Surface pores of corn starch can form centres of enzymatic attack [18]. According to them the presence and number of pores and fractures depends on botanical origin of starch. The occurrence of pores on potato starch surface was reported by Sterling [24]. Gallant et al. [10] stated, that under alpha-amylase treatment corn starch granules are much more frequently eroded than those of potato, which can be associated with the number of pores. Fannon et al. [6] observed pores on the surface of starch granules from potato, tapioca, oat and rice. They also assumed that the observed pores are a natural morphological feature of the granules and affect their susceptibility to enzymatic hydrolysis and chemical reactivity. In further studies [7] it was found, that the diameter of pores varies from 100 to 300 nm. The same authors found that some of surface pores are the outlets of internal channels penetrating the granules to the hilum, which are 70-100 nm in diameter. The presence of internal channels in starch granules of sorgo and corn was confirmed by Huber and BeMiller [13, 14].

In the studies on surface structure of starch granules atomic force microscopy was used among other tools. Thomson et al. [25] observed that the susceptibility to enzymatic hydrolysis of different granules isolated from one source varies. If the surface is without fractures, cracks and pores the degree of erosion caused by enzyme is very low. If there are pores or fractures, they facilitate action of the enzyme. The next studies using AFM [4, 5] revealed the occurrence of surface structures 10-300 nm in diameter on potato starch granules. They could be divided into two main types: smaller ones, which were 10-50 nm in diameter and larger ones, 50-300 nm in diameter. Wheat starch exhibited much smoother surface, visible structures were 10-50 nm in diameter. It is supposed that these structures are of carbohydrate origin and form external parts of amylopectin clusters, which is strictly connected to blocklet model of starch granule proposed by Gallant et al. [9]. Ohtani et al. [20] mentioned that an average size of the observed structures is about 30 nm and that they could be found on the surface of starch granules from corn, potato, rice and wheat. They also proved that such structures may form larger aggregates. AFM microscopy was also used to studying internal structure of starch granules [2, 22]. Baker et al. [2] observed the well-known radial organization of starch macromolecules, with a less-ordered hilum region near the centre. They noticed blocks 400 – 500 nm in size that span the growth rings.

Figures 7 and 8 demonstrate so called height profiles of surface fragments of triticale starch granules. Each line A and B which are displayed on figures corresponds to a section shown below, which demonstrates the arrangement of granule's surface. The part of granule shown on Figure 7 contains some pores visible as black spots. Although the measured height difference is only 12 nm it can not be regarded as a real depth of the pores. Due to methodology it is impossible to distinguish between typical surface pores and outlets of deeper channels which can penetrate the granule to the hilum. Brighter areas can be formed by surface structures 150-200 nm in sizes consisting of smaller structures already observed by other authors [4, 5, 20]. According to Ohtani et al. [20] surface structures on starch granules which are approximately 30 nm in diameter can build up larger agglomerates.

Fig. 7. Height profiles of triticale starch granule surface (image size 0.6 x 0.6 μm, height difference 12 nm)

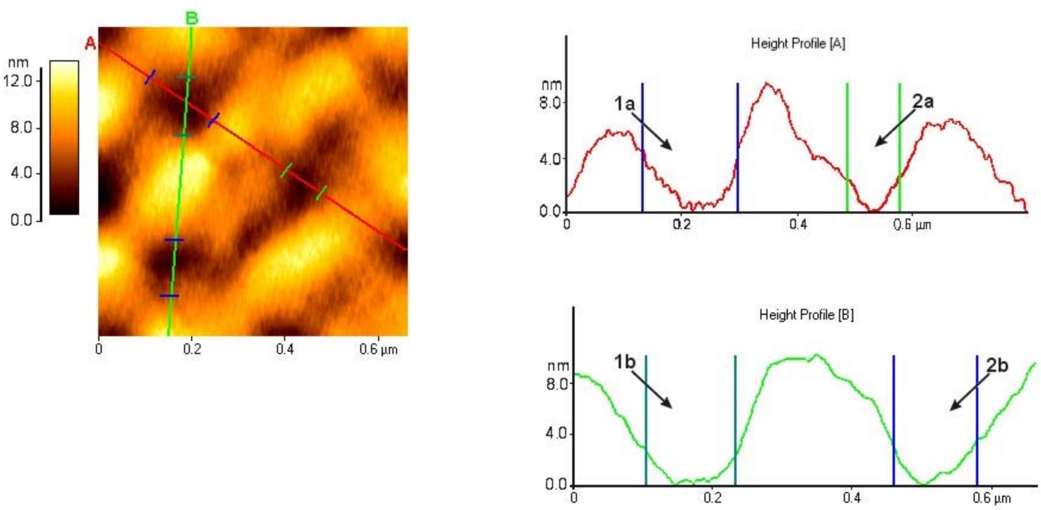
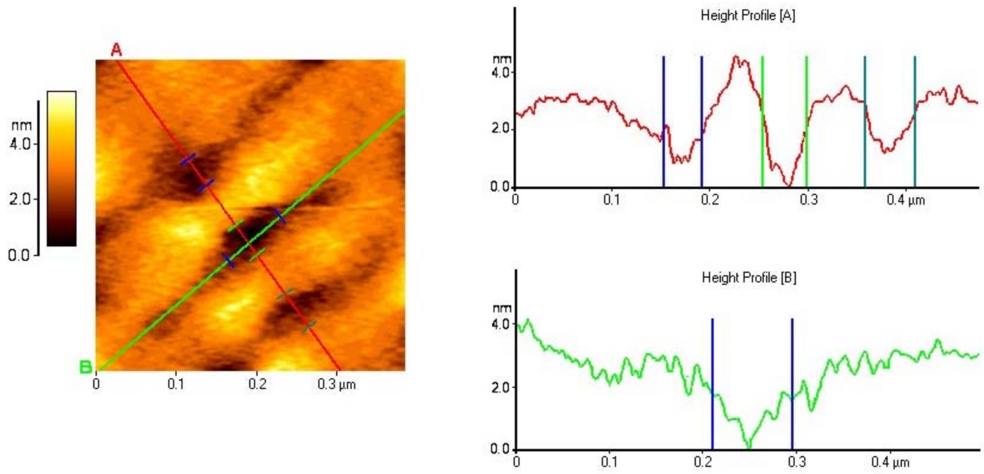


Fig. 8. Height profiles of triticale starch granule surface (image size 0.4 x 0.4 µm, height difference 4 nm)



Height profile A (Fig. 7) demonstrates section of granule's surface with marked diameters of the observed pores: arrow 1a-165 nm, arrow 2a-92 nm. The diameters of pores visible on height profile B are: 1b-128 nm and 2b-120 nm. The pores have regular shape, with the exception of the one marked as 2a (height profile A), where the central part visible as the darkest area is surrounded by brighter domain with slightly elongated shape. Figure 8 demonstrates height profiles of other surface part of triticale starch granule. This fragment seems to be smoother, however three oval pores are clearly visible. Their dimensions (profile A, arrows 1a, 2a, 3a) are close to 40-50 nm, while the diameter 1b (height profile B) equals 85 nm. The observation of pores over 100 nm in diameter is in agreement with earlier studies by Fannon et al. [7] on other starch types. The measurements shown on height profiles on the fragment of granule's surface (Fig. 8) imply that they can be also smaller. Certainly, such a measurement is subjective, because of the absence of absolute reference point. It is difficult to say if the diameter should be measured in half of pore's depth or between highest points in its vicinity.

Fig. 9. NC-AFM images of triticale starch granule surfaces. The scan sizes and height differences' are respectively: a) 0.6 x 0.6 µm, 12 nm; b) 0.4 x 0.4 µm, 4 nm; c) 2 x 2 µm, 20 nm; d) 0.8 x 0.8, 8 nm; e) 1.2 x 1.2 µm, 12 nm; f) 0.6 x 0.6 µm, 8 nm; g) 0.8 x 0.8 µm, 8 nm; h) 0.6 x 0.6 µm, 8 nm

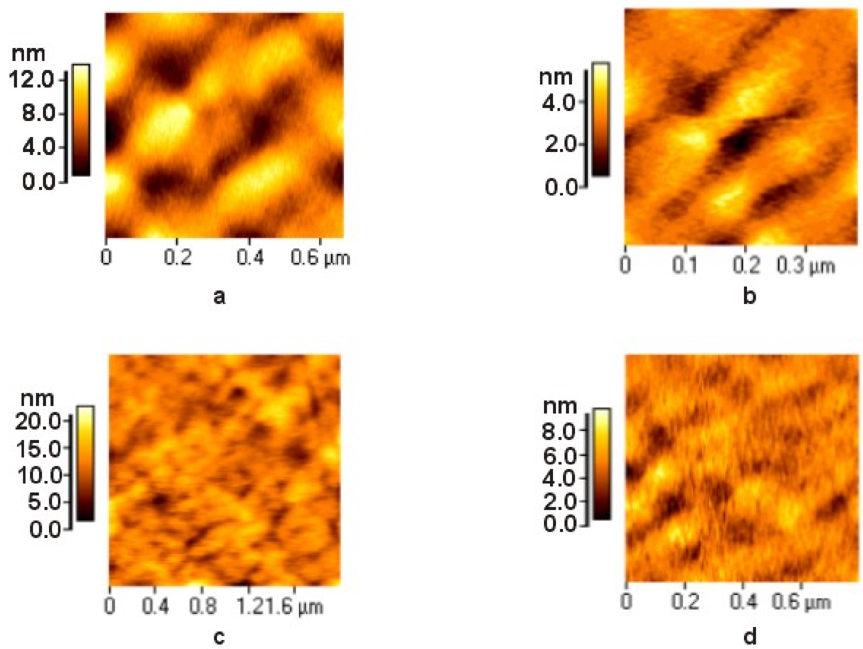


Fig. 9. cont.

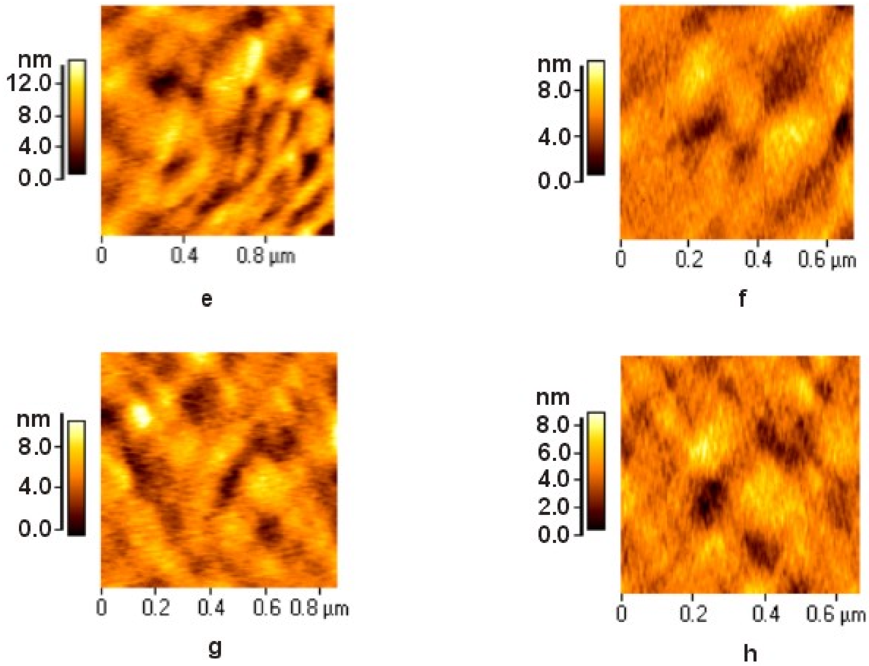


Figure 9 represents other surface parts of triticale starch granules. The surface structures 50-100 nm in diameter, can be observed as lightest regions (Fig. 9 c, e, f), pores and holes are seen as black spots. They can have relatively regular, oval shape (Fig. 9 b, c), form fuzzy areas (Fig. 9 d, f) or be elongated or slit-like (Fig. 9 c, e). Surface part shown on photo 9d is relatively flat and smooth without visible pores and surface structures. According to Fannon et al. [6, 7] such entities can be a natural morphological feature of starch granules. Some of them may be outlets of channels penetrating starch granule to hilum [13, 14]. Other, especially slit-like, may be created during milling of triticale grains or other processes related to isolation and drying of starch [3]. In case of triticale starch, it is also possible that the observed pores and structures present on surface of starch granules may appear due to the action of amylolytic enzymes, which are abundant in the endosperm of this cereal.

CONCLUSIONS

Basing on the observations made by means of atomic force microscopy (NC-AFM) it can be concluded, that surface of triticale starch granules contains both holes or pores and minor surface structures. The occurrence of pores above 100 nm in diameter is in agreement with earlier studies on other starch types, smaller pores present on surface of triticale starch granules have not yet been reported. Such holes may have regular-oval or circular shape or exist as slit-like objects. Holes or pores and surface structures are situated quite irregularly. Visible surface structures below 200 nm in diameter are probably aggregates of smaller units. Parts of smooth, flat surface with no visible pores and surface structures have also been observed.

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