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Characterising Starch Granule Surfaces in Durum Wheat Using Atomic Force Microscopy

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Abstract. *Durum wheat is used for making pasta and semolina. Knowledge of the structure and properties of microscopic surfaces of durum wheat starch granules at the nanometer level is essential for understanding the functional and physico-chemical properties. The influence of the nanoscale nature of the surface undulations on the starch granules inside durum wheat is macroscopically relevant as it affects the milling properties. The objective of this study is to visualize the size and dispersion of the starch grains and the structure of the binder material in wheat using atomic force microscopy. We examined and compared the distribution of starch granules at the nano level in vitreous and non-vitreous durum wheat kernels using atomic force microscopy images.*

Keywords. Starch, granule surface, blocklets, atomic force microscopy

Introduction

The annual world production of durum wheat is about 26 million tonnes (USDA, 2005). Durum wheat is used for making semolina and pasta. One of the important factors in determining the quality of durum wheat is vitreousness. Vitreous durum wheat kernels are glassy and translucent in appearance while non-vitreous kernels contain a starchy or mottled appearance.

Kernel vitreousness is associated with semolina granulation, color, and protein content. The less vitreous the kernel, the finer the granulation and the lower the color and protein content. Kernels that are less vitreous will produce more flour thus resulting in less semolina product. Generally, vitreous kernels are harder in nature and non-vitreous kernels are softer. Kernel hardness also affects the water absorption capacity of wheat flour which is directly related to the amount of bread produced from a given weight of flour (Dexter and Marchylo, 1996). Hard wheat has vitreous endosperm which requires more energy to grind while soft wheat is easier to grind. For bread making, soft and non-vitreous kernels are preferred because of the higher water absorption. Glenn and Saunders (1990) demonstrated that intracellular space exists around the starch granules of soft, but not hard wheat, forming a discontinuity in the starch-protein mix.

Starch granule size distribution and the total volume occupied by starch granules contribute significantly to the rheological properties of wheat flour dough (Edwards et al., 2002). The influence of nanoscale nature of the surface undulations on the starch granules inside durum wheat is macroscopically relevant as it affects the milling properties. The size and distribution of starch granules becomes important, when aiming at functional and physio-chemical properties. Morphology and topography of surface is an important feature of solids used as raw material (Aguilera, 2000). Hence, there is a need to measure the size and distribution of starch granules of durum wheat. Measurement of size and the analysis of starch granules will give information to understand the difference in functional properties of the vitreous and non-vitreous kernels during milling.

Atomic Force Microscopy (AFM) is a powerful, revolutionary technique that enables us to analyze surface features up to the atomic and molecular level. Employment of AFM in food and cereal science research is emerging at a rapid pace. Morris et al., (2001) have proven that AFM is the best tool for interpreting rheology of food biopolymers at the molecular level. AFM has been used to image the starch granule surfaces of barley, oat, wheat and maize (Juszczak et al., 2003, Ohtani et al., 2000). AFM has also been used to image bacteria of different taxonomy groups (Bolshakova et al., 2004) and roughness of peach during controlled atmosphere storage (Yang et al., 2004).

Principle of Atomic Force Microscopy

The atomic force microscopy was developed by Binnig et al. (1986). AFM creates an image by scanning a sharp stylus which is attached to a flexible cantilever, across the sample surface (Figure 1). The atomic force microscope captures images by feeling the detail on the surface of the specimen. When the stylus is held in extreme close proximity to the sample, repulsive force comes into play, causing the cantilever to bend away from the surface. By monitoring the cantilever bending as the cantilever-stylus assembly is scanned over the sample surface, any undulations in the sample can be recorded. This system is highly sensitive as it can detect cantilever deflections produced by the stylus scanning over individual atoms and molecules.

Though the atomic force microscope looks simple, it is extremely complex in its functionality. The force exerted on the sample by the stylus is important in determining the level of contrast in the image. If too low a force is applied, the stylus mistracks and the contrast will be poor. At the same time a higher force will poke through the sample causing distortion or damage to the sample. By mounting the cantilever-stylus assembly on to a piezo electric device, the distance between the stylus (tip) and the sample can be automatically adjusted with respect to the extent of cantilever bending. The precision with which a piezoelectric device can be used to position the probe relative to the surface is the key to the high resolution of the atomic force microscope. Contact mode and error signal mode are the two modes of imaging the sample surfaces using atomic force microscopy. Besides probing surface morphology and surface forces, AFM makes it possible to characterize the local mechanical properties of the sample.

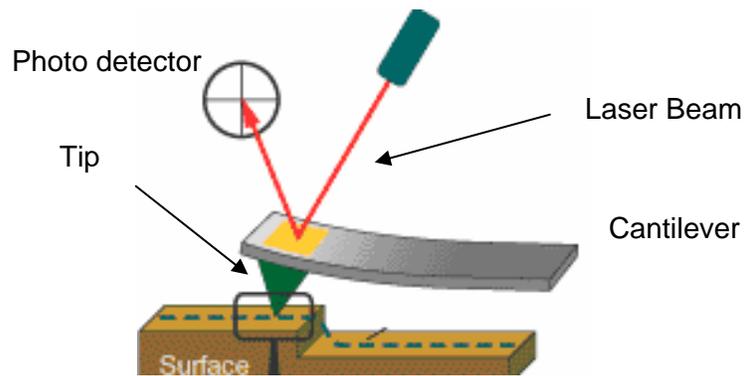


Figure 1. Schematic diagram showing the working principle of an atomic force microscope.

The objectives of this study were:

- 1) to visualize the size and dispersion of the starch grains of vitreous and non-vitreous durum wheat using atomic force microscopy, and
- 2) to compare the starch granule surface differences of vitreous and non-vitreous durum kernels.

Materials and Methods

Samples

Durum wheat kernel (*Triticum turgidum* L. var. *durum*) samples collected from terminal elevators at Thunder Bay, Canada were used in this study (Figure 2). Kernels were manually separated into vitreous and non-vitreous sets based upon a visual assessment. Care was taken to avoid the weathered and bleached kernels in the sample set. Starch from the vitreous and the non-vitreous kernels were prepared by the following steps: 1) dough preparation, 2) dough tempering, 3) elution of starch using 0.1% aqueous NaCl solution, 4) centrifugation of starch suspension, 5) elimination of supernatant and impurities, 6) starch drying (freeze drying), 7) starch lumps disintegration and 8) starch sieving. Starch prepared from both the vitreous and non-vitreous kernels was collected separately in two different plastic bags.

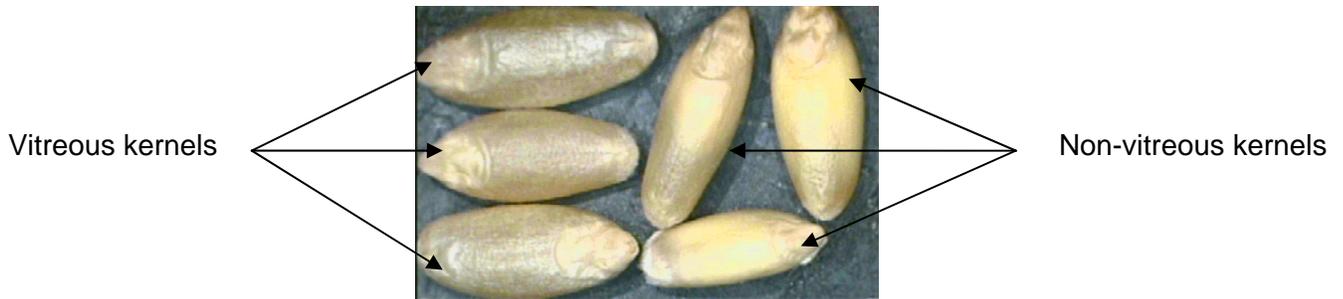


Figure 2. Photographic image of vitreous and non-vitreous durum wheat kernels.

Sectioning using a microtome

A small quantity of starch sample was placed in the bottom of a plastic beam capsule and embedded in fresh Spurr epoxy resin. Using a pointed wooden stick, the starch sample was slightly stirred allowing any air bubbles to escape. After 15 minutes of settling, the beam capsules were polymerized in an oven at 700° C for 12 hours. Samples were carefully removed from the plastic beam capsules and placed directly into the microtome chuck. The block face was polished using a Reichert-Jung Ultracut microtome. 0.5 micron smoothness was achieved at the exposed surface of the resin using a fresh dry glass knife. Once the block face was cut, the block was removed and the sample was ready for imaging. Both the vitreous and non-vitreous starch samples were embedded in the resin in the same fashion. A sample beam was also prepared with only the Spurr epoxy resin for reference imaging.

Imaging

A Dimension 3100 AFM (Digital Instruments, Santa Barbara, USA) was used to image the samples. Imaging was performed in the contact mode with silicon nitride probes mounted on cantilevers with normal spring constants of 0.06 – 0.12 N/m. Scan areas varied between 100 X 100 μm and 1X1 μm . Observation of starch granule surface on the vitreous starch samples, non-vitreous starch samples and the pure sample beam were scanned using AFM and an Olympus BX51 optical microscope (Olympus Optical Company, Tokyo, Japan).

Results and Discussion

Figure 3 shows the AFM images of pure resin samples. Figure 4 shows the atomic force microscopic images of vitreous and non-vitreous durum granules at different scan size areas. The comparison of the images from figure 3 and figure 4 proves that the granules observed in the figure 4 are starch grains and easily distinguished from sectioning artifacts. The granules can be distinguished from the surrounding resin due to the knife marks during microtome sectioning. From figure 4 (b), one can observe that there is a definite difference in contrast between the granule and the encasing resin. As a reference checking, the sample beams embedded with vitreous and non-vitreous starch granules were also imaged with optical microscope. From the images (Figure 5), it is observed that the starch granules are larger in size in the non-vitreous image than in the vitreous image.

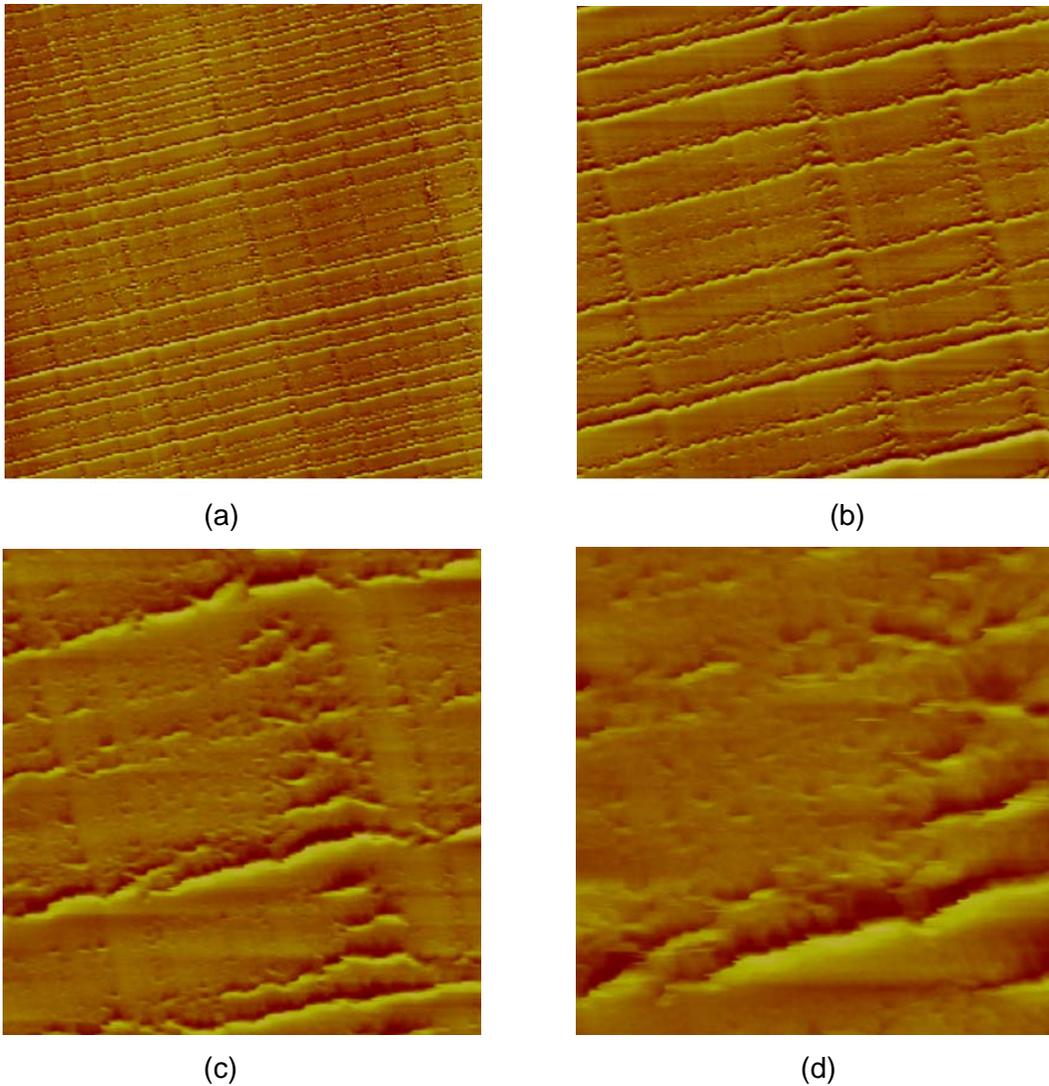
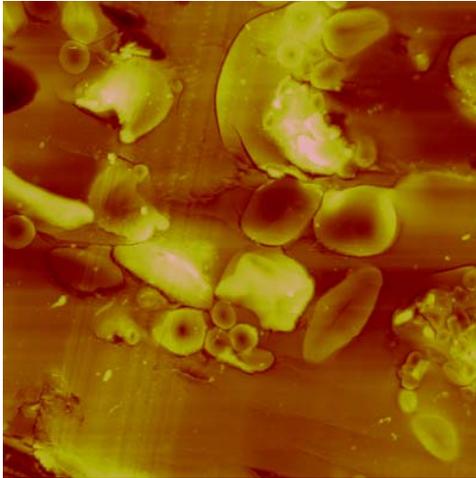


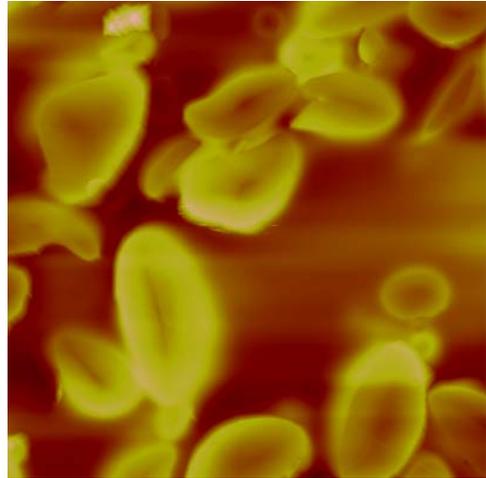
Figure 3. AFM images of pure spurr resin samples. Scan size areas of the images are (a) 80 X 80 μm , (b) 30 X 30 μm , (c) 10 X 10 μm and (d) 5 X 5 μm .

The area occupied by the starch granules in the vitreous and non-vitreous kernel images was calculated by measuring the individual areas of the starch granules. The total area of starch granules in the non-vitreous images was 1.65 times greater than the starch granules area of the vitreous type. This shows that the starch granules are larger and more in number in the non-vitreous type compared to the vitreous type.

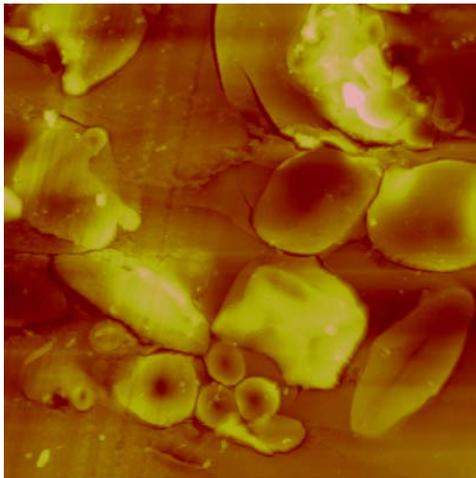
In all the images, starch granules are elongated in shape. Diameter of the starch granules in non-vitreous images is bigger than the vitreous images.



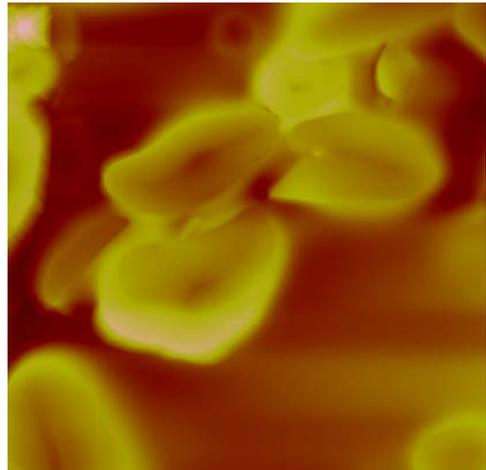
(a)



(b)

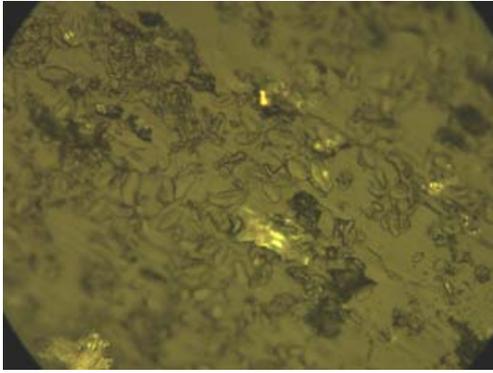


(c)

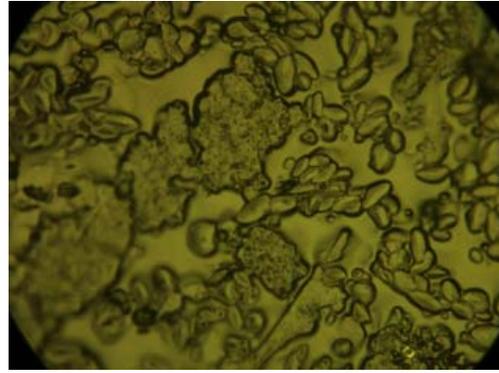


(d)

Figure 4. AFM images of durum wheat starch granule surfaces. (a), (c) are vitreous starch granules and (b), (d) are non-vitreous starch granule images. The scan size areas are 80 X 80 μm for images (a), (b) and 50 X 50 μm for images (c) and (d).



(a)

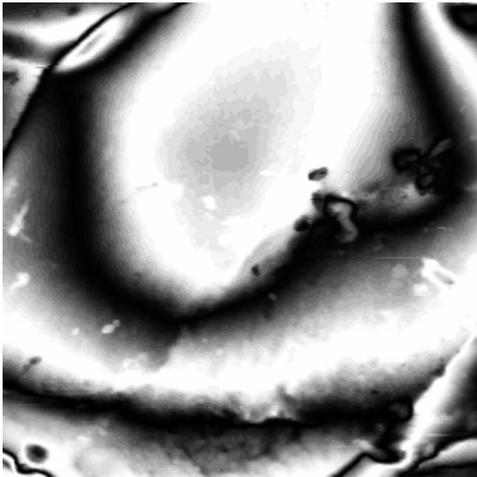


(b)

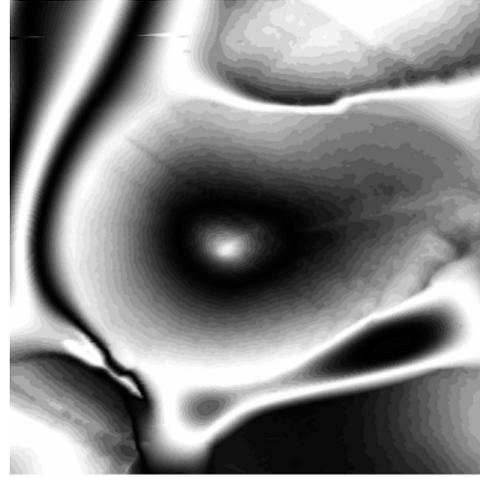
Figure 5. Optical microscope images of exposed starch granule surfaces embedded in spurr epoxy resin at 50X resolution (a) vitreous kernel starch granules, (b) non-vitreous kernel starch granules.

Growth Rings

The topographic image of the non-vitreous starch granule (Figure 6) clearly shows the growth rings but is not clearly visible in the vitreous starch granule surfaces. The comparative appearance of the granules between non-vitreous and vitreous shows that AFM provides interesting information about the growth rings.



(a)



(b)

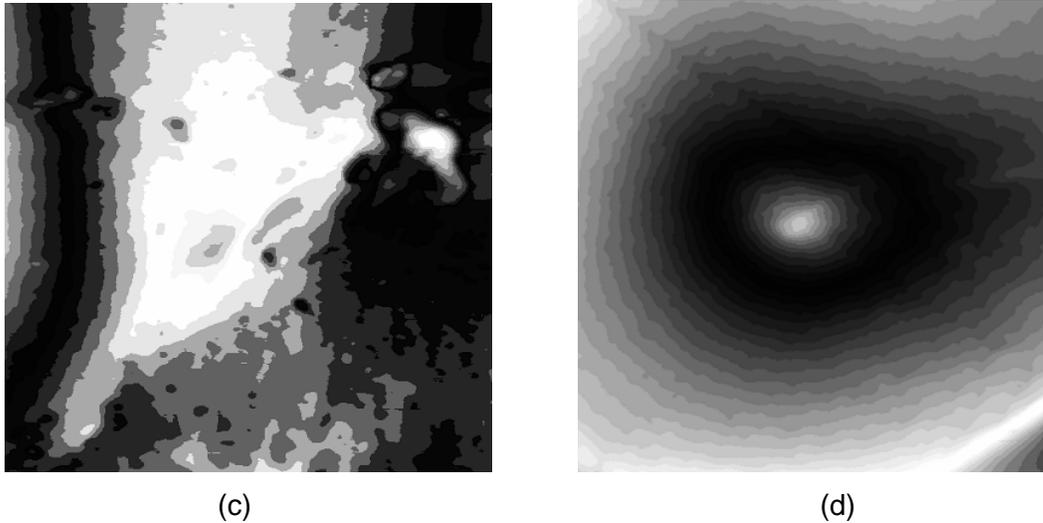


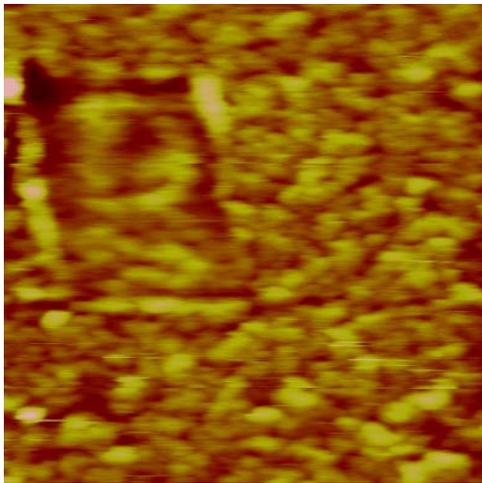
Figure 6. AFM image of starch granule surfaces showing growth rings. (a), (c) are vitreous starch granules and (b), (d) are non-vitreous starch granule images. The scan size areas are 10 X 10 μm for images (a), (b) and 5 X 5 μm for images (c) and (d).

Generally starch consists of two types of molecules, amylose and amylopectin. The relative proportion of amylose to amylopectin depends on the source of the starch (Singh et al., 2003). Amylopectin molecules of the starch generate concentric layers that contribute to the growth rings of the starch (Parker and Ring, 2001). Our observation of growth rings explains that non-vitreous kernels might have more amylopectin molecules than the amylose molecules.

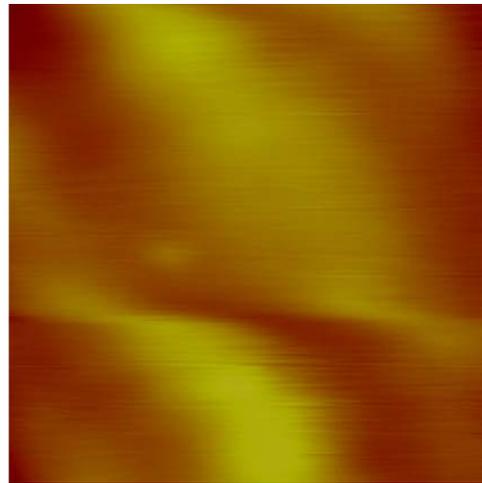
Buttrose (1962) proposed that environmental factors influence the growth ring formation of the starch granules in cereals. The degree to which wheat becomes non-vitreous, is decided by factors such as weather conditions and soil fertility (Phillips and Niernberger, 1976). Observation of growth rings on the non-vitreous starch granules reveals the fact that environmental conditions predominantly determine the vitreousness nature of the durum wheat.

Ultrastructure of granules

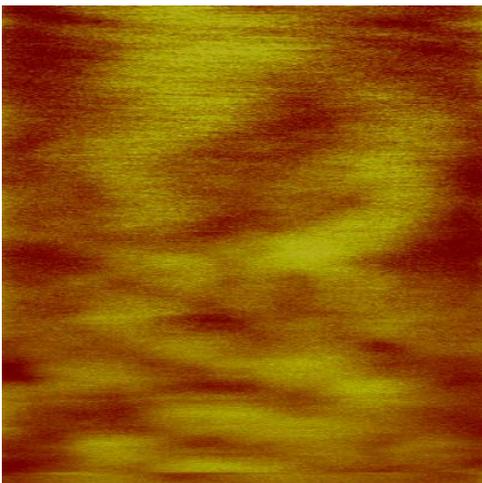
The AFM images of the starch granule surfaces at higher resolution shows that the surface is rough with a random pattern of features with an approximate size of 100 nm. Inspection of the banding pattern observed in the samples at higher resolution (Figure 6), reveals that the dark bands are sometimes discontinuous. Bright and dark banded blocklet structures are clearly visible from the figures 7(b) and 7(c). This is consistent with the blocklet model proposed in a previous study (Ridout et al., 2002).



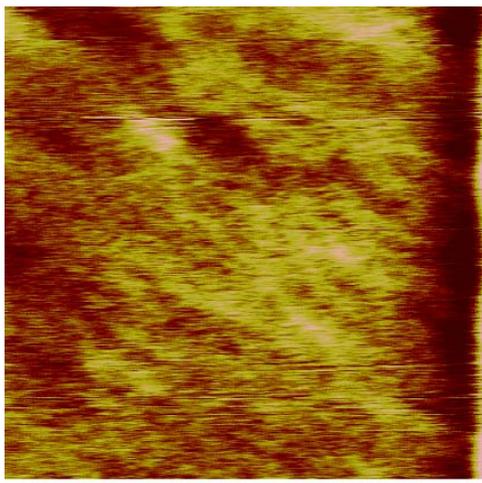
(a)



(b)



(c)



(d)

Figure 7. AFM images of surface details of sections of durum wheat starch encased in spurr epoxy resin revealing banding pattern and blocklets. (a), (c) are vitreous starch granule surfaces and (b), (d) are non-vitreous starch granule images. The scan size areas are 3 X 3 μm for images (a), (b) and 1 X 1 μm for images (c) and (d).

Conclusions

Based on the results of our study, the following conclusions can be drawn:

1. AFM is a powerful tool to study the ultrastructure of starch granules.
2. Our study confirms the 'blocklet model' of the ultrastructure of starch granule surface.
3. Starch granules were larger in size and more in number in the non-vitreous type than the vitreous type. Growth rings were observed only in the non-vitreous type.

4. Based on the observation of growth rings, and the number of starch granules in the non-vitreous type, it is proposed that the non-vitreous durum wheat starch has more amylopectin molecules than amylose.

Acknowledgements

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Reference:

- Aguilera, J.M. 2000. Microstructure and food product engineering. *Food Technology* 54(11): 56-65.
- Binnig, G., C.F. Quate, and C.H. Gerber. 1986. Atomic force microscope. *Physical Review Letters*. 56: 930–933.
- Bolshakova, A., O. Kiselyova and I.V. Yaminsky. 2004. Microbial surfaces investigated using atomic force microscopy. *Biotechnology Progress* 20: 1615-1622.
- Buttrose, M. S. 1962. The influence of environment on the shell structure of starch granules. *Journal of Cell Biology* 14: 159–167.
- Dexter, J.E. and B.A. Marchylo. 1996. Meeting the durum wheat quality requirements of an evolving processing industry: past, present and future trends. In *Pavan Mapimpianti 50th anniversary durum wheat and pasta seminars*, Bassano del Grappa, Italy October 23-26.
- Edwards, N.M., J.E. Dexter and M.G. Scanlon. 2002. Starch participation in durum dough linear viscoelastic properties. *Cereal Chemistry* 79(6): 850-856.
- Glenn, G.M. and R.M. Saunders. 1990. Physical and structural properties of wheat endosperm associated with grain structure. *Cereal Chemistry* 67: 176-182.
- Juszczak, L., T. Fortuna and F. Krok. 2003. Non-contact atomic force microscopy of starch granules surface Part II. Selected cereal starches *Starch/Starke* 55: 8 – 18.
- Morris, V. J., A.R. Mackie, A. R. Wilde, P. J. Kirby, A. R. Mills and A.P. Gunning. 2001. Atomic force microscopy as a tool for interpreting the rheology of food biopolymers at the molecular level. *Food Science & Technology* 34: 3-10.
- Ohtani, T., T. Yoshino, S. Hagiwara, T. Maekawa and T. Tsukuba. 2000. High resolution imaging of starch granule structure using atomic force microscopy. *Starch/Starke* 5: 150-153.
- Parker, R. and S. G. Ring. 2001. Aspects of the physical chemistry of starch. *Journal of Cereal Science* 34: 1-17.
- Ridout, M.J., A.P. Gunning, R. H. Wilson, M.L. Parker, and V.J. Morris. 2002. Using AFM to image the internal structure of starch granules. *Carbohydrate Polymers* 50: 123–132.
- Phillips, D.P. and F.F. Niernberger. 1976. Milling and baking quality of yellow berry and dark, hard and vitreous wheats. *Bakers Digest* 50(2): 42-48.

- Singh, N., J. Singh, L. Kaur, N.S. Sodhi and B.S. Gill. 2003. Morphological, thermal and rheological properties of starches from different botanical sources. *Food Chemistry* 81: 219-231.
- USDA. 2005. Production Estimates and Crop Assessment Division. United States Department of Agriculture.
- Yang, H., G. Feng, and Y. Li. 2004. Investigating the roughness of peach during controlled atmosphere storage by atomic force microscopy. ASAE Paper No. 046186. St. Joseph, MI: ASAE.