P-064	**Nano-structure of a starch-based hydrogel, crosslinked by Concanavalin A**

Kesselman E. [#]**, Shazman A., and Shimoni E.** [*]
Biotechnol & Food Eng, Technion – Israel Institute of Technology, Haifa, Israel.
* Supervisor # Contact email: ellina@tx.technion.ac.il

INTRODUCTION AND OBJECTIVES

The ability of starch molecules to associate in an aqueous medium and to create three-dimensional networks, i.e. gelation of starch, is an essential property used in food, pharmaceutical and agricultural applications. Starch hydrogels can be formed by physical interactions between starch polysaccharide chains, or by covalent crosslinking (Biliaderis 2009).

In the current project we study a new concept of forming starch gels: by a lectin-based specific crosslinking. Microstructure of these gels determines their physical and mechanical properties, enables the development of a controlled drug delivery and other important applications.

This work is aimed to characterize the microstructure of starch hydrogels crosslinked by a lectin using several microscopy techniques.

MATERIALS AND METHODS

The gels were formed in a three-component system: amylopectin (of Waxy starch), concanavalin A (a lectin from jack beans) and ions Mg^{2+} and Ca^{2+} as salts essential for binding of the lectin to the sugar.

Light microscopy (LM), Atomic Force Microscopy (AFM), Confocal Laser Scanning Microscopy (CLSM), Cryo-Transmission Electron Microscopy (Cryo-TEM), Scanning Electron Microscopy (SEM) and High-Resolution Cryo-SEM (HR-Cryo-SEM) were used to characterize the gel microstructure. Control systems, lacking one or two components, were also characterized.

RESULTS AND DISCUSSION

The results obtained by LM and CLSM demonstrated that amylopectin / Concanavalin A/salts gels had very smooth structure, and Concanavalin A was distributed homogeneously in them.

Cryo-TEM revealed difference in the nano-structure of amylopectin in the absence and in the presence of salts. Salts caused separation of amylopectin chains and formation of network-like structure in the nanometric scale.

Using AFM, nanostructure of the gels and their components was imaged at a lateral resolution less than 5 nm, and a height resolution less than 1 nm (Figure 1). 10×10-µm scan (A) showed smooth and very low, nm-height topography. Amylopectin-consisting samples revealed

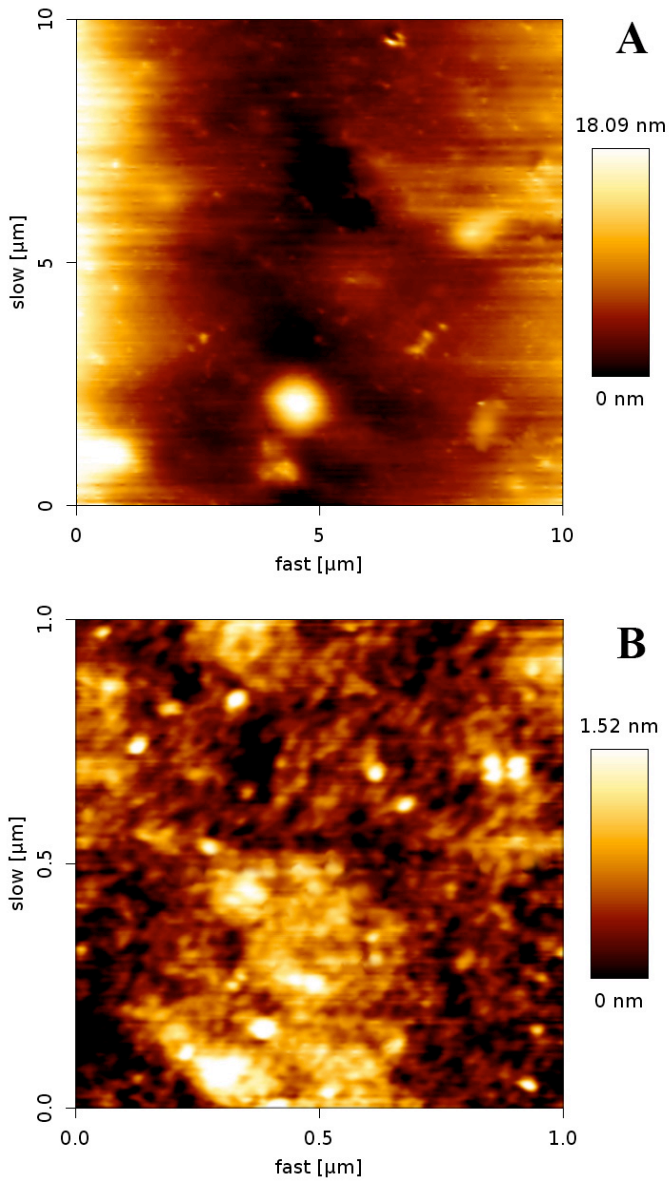

Figure 1: AFM of dried 8% amylopectin gel, crosslinked with Concanavalin A in the presence of salts, imaged in the intermittent contact mode in air.
(A) 10×10-µm scan; (B) 1×1-µm scan.

multiple small nanometric-size protrusions on their surface, seen clearly at 1×1-µm scan area (B). The protrusions had approximately 20 nm of width and a few angstroms of height, and looked like organized biopolymer molecules. These features could be proteins, originated from starch biosynthesis enzymes, remained in the amylopectin, and phase-separated to the surface during gelation process (Rindlav-Westling 2003). Alternatively,

there could be crystalline units of the retrograded amylopectin, possessed similar dimensions (Putaux 2000).

SEM of freeze-dried gels showed high porosity at the range of tens of micrometers (Figure 2). These large pores were not seen neither in LM- and CLSM-images of the original wet gel, nor in AFM scans, and most likely were artifacts of the drying technique, which is not fast enough to prevent structure alterations.

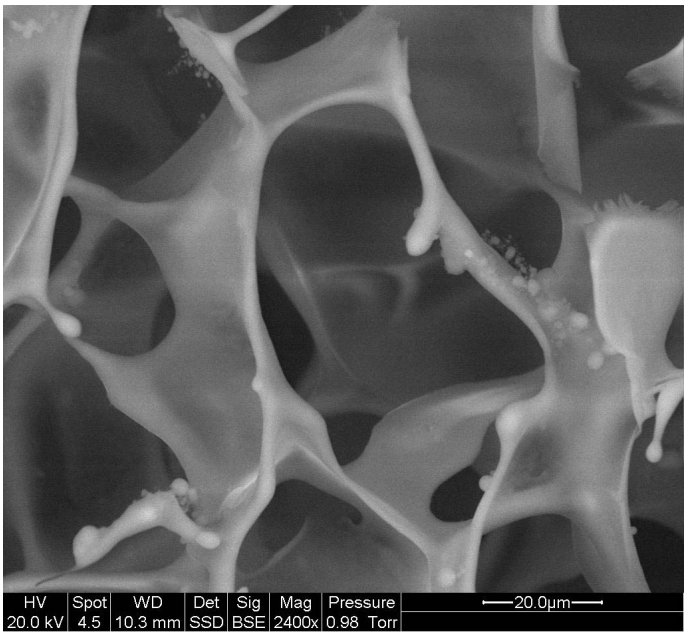

Figure 2: SEM of freeze-dried gel Amylopectin/Concanavalin A/salts.

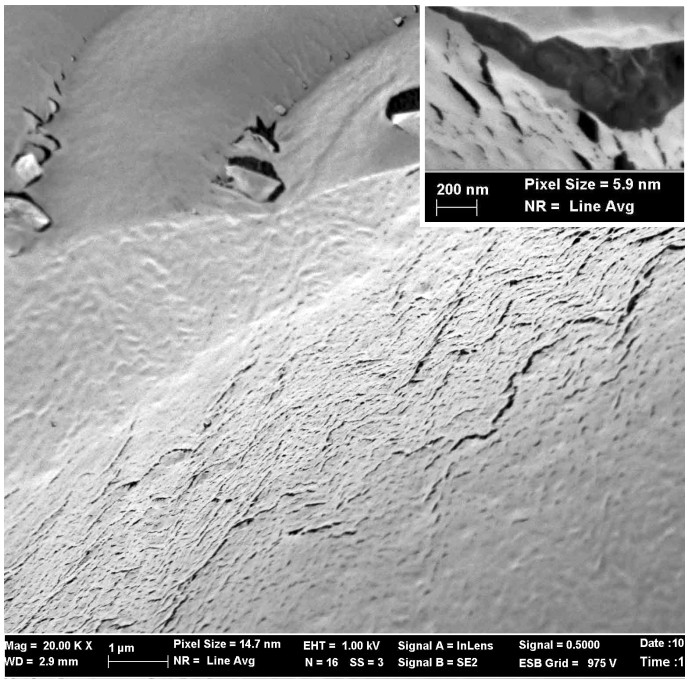

Figure 3: HR-Cryo-SEM of vitrified and fractured gel Amylopectin/Concanavalin A/salts at the magnification 20 000×. In the insert the magnification is 50 000×.

HR-Cryo-SEM of a vitrified in liquid ethane and fractured gel revealed smooth surface, no porosity, and in some places fine fiber-like pattern, exhibited better when irradiated at a high magnification (Figure 3). Left upper and right lower corners of the image show flat and smooth surface. At the slope (center) a fiber-like pattern is seen. Imaging at the magnification 50 000× (insert) revealed tiny nm-range porous structure under the gel fracture surface, with pore diameter ~ 100 nm, and wall thickness ~ 20 nm.

These results are correspondent to those obtained by LM, CLSM and AFM, and vote for the homogeneous gel, structured in nanometric dimensions.

CONCLUSIONS

The results demonstrated that amylopectin hydrogels, crosslinked by Concanavalin A in the presence of Mg^{2+} and Ca^{2+} salts possessed a continuous and homogeneous structure up to the nanometric scale. This indicates creation of the gel, highly ordered at the molecular level.

Dimensions of the nano-scale pores, demonstrated by Cryo-HR-SEM, corresponded to the amylopectin network dimensions, revealed by Cryo-TEM.

REFERENCES

- Biliaderis C. G. (2009). In: *Starch: Chemistry and Technology*, Eds: J. N. BeMiller, R. L. Whistler, 3rd ed., Food Science and Technology, International Series, Elsevier, USA, 332-353.
- Rindlav-Westling A. and Gatenholm P. (2003) *Surface Composition and Morphology of Starch, Amylose, and Amylopectin Films.* Biomacromolecules, 4(1) 166-172.
- Putaux J.-L. et al. (2000) *Network formation in dilute amylose and amylopectin studied by TEM.* Macromolecules, 33, 6416-6422.