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Effects of heating modes and sources on nanostructure of gelatinized starch molecules using atomic force microscopy

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Abstract

Potato and corn starches were subjected to convective and microwave heating. The effects of microwave heating on nanostructure of starch molecules were studied by atomic force microscopy (AFM). Potato starches formed networks with the height from 0.3 to 11.0 nm under microwave radiation. Chains were observed dissociated from the networks with a nanoparticle head. Starch chains can be rod-like conformations or thinner linear structures on nanometer scale. Rod-like chains were about 1.0 nm in height, while the thinner chains were about 0.3 nm. However, corn starches did not show any networks under microwave processing. The capped chains of corn starches were similar to those of potato starches. The results revealed that microwave heating caused incomplete gelatinization of starch by comparison with convective heating. Heating modes influence the potato starch much than that on corn starch. The results can be applied on material selection for microwaved food development.

Keywords: Nanostructure; Atomic force microscopy (AFM); Microwave; Potato starch; Corn starch

1. Introduction

Microwave-assisted technologies are used extensively for thermal processing of water-containing foodstuffs, for example, heating, drying, extraction, sterilization, thawing, dehydration, and age accelerating (Chua, Mujumdar, & Chou, 2003; Feng, Tang, Mattinson, & Fellman, 1999; Khraisheh, McMinn, & Magee, 2004; Nijhuis et al., 1998; Oliveira & Franca, 2002; Wang, Wig, Tang, & Hallberg, 2003). In starch industry, microwave is often used for heat-moisture treatment (Anderson & Guraya, 2006) and catalysis of chemical modification(Liu, Liu, & Kennedy, 2005) by the volumetric rapid heating. The structural change of starch due to microwave may relate to many of its quality problems, such as changes in color and texture, case hardening, and shrinkage. Along with these interests, the structural properties of microwaved starch are of crucial importance. As the main carbohydrate of food, starch participates in the Maillard reaction and pyrogenetic reaction during microwave heating. These reactions might be closely related to their conformational change at nanometer level.

Recent studies indicated that texture of starch from different resources was responsible for their behavior under frequency radiation. Lewandowicz, Fornal, and Walkowski (1997) reported that the crystal and structural state of potato starch had been changed after microwave radiation. Waxy corn starch and normal corn starch had been demonstrated to have distinct responses to microwave heating (Lewandowicz, Jankowski, & Fornal, 2000). The results of waxy or non-waxy rice starch also proved that the content of amylose influenced its physico-chemistry properties (Anderson & Guraya, 2006). However, few works talk about the effect of microwave radiation on structural change of starch at molecular level. Studies of phenomena such as shrinkage, puffing, and stress cracking are challenging for researchers in this area.

Atomic force microscopy (AFM) offers an opportunity to image and manipulate single biological macromolecules

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with high resolution (An, Guo, Zhang, Zhang, & Hu, 2005; Round, MacDougall, Ring, & Morris, 1997; Yang, An, & Li, 2006; Yang, Wang, Lai, An, Li, & Chen, 2007). In our previous works, AFM had been successfully used for study of pectin extraction (Liu, Wei, Guo, & Kennedy, 2006; Yang, An, Feng, & Li, 2005; Yang, An, Feng, Li, & Lai, 2005), membrane formation of main rezin (Guo, Liu, An, Li, & Hu, 2005), and catalysis of starch modification (Liu et al., 2001).

The object of this paper was to investigate the effects of different heating modes on the nanostructure of starch molecules. The results would help in revealing the causes of quality losses like changes in color, shrinkage, and texture and also in food materials selection for microwaved food development.

2. Experimental

2.1. Gelatinization of starch

Commercial potato starch (amylose content 20%) or corn starch (amylose content 26%) (Dingxin starch plant, Tianjin, China) was mixed with double-distilled water (0.1 mg/ml) in a covered vial and stirred for 2 min. For convective heating method, the starch was gelatinized in boiling water bath for 5 min. For microwave heating, the covered vial was put into microwave oven (Galanz) with a 2450 MHz microwave frequency under 750 W. After the water boiled, the system heating continued for 5 min.

2.2. Manipulation of starch molecules

The solution of the gelatinized starch (5 μl) was dropped onto a newly cleaved mica surface by a pipette. The starch molecules were manipulated by a method named "fluid fixation" technique (Wang, Lin, & Schwartz, 1998; Yang, An, & Li, 2006), which is used for DNA and pectin manipulations. Then the sample was blown dry with clean air and was ready for AFM investigation.

2.3. AFM imaging

Imaging of starch was performed with a Multimode AFM (Nanoscope IIIa, Veeco Instruments, Santa Barbara, CA, USA) equipped with a J or E scanner. Silicon cantilevers (NSC-11, MikroMasch) with a force constant of 48 N/m (cantilever A) were used. All operations were carried out in air at room temperature inside a glove box under relative humidity of 25–35%, which was controlled by silica gel (Yang, Lai, An, & Li, 2006). Images were collected by the Tapping Mode AFM. The bright areas in AFM images correspond to peak features in height. All the AFM images were presented without any image processing. Statistics of chains and particles were obtained by image J software.

2.4. Statistical analysis

The AFM images were analyzed offline with image J software. It should be noted that different scales were used in the vertical and horizontal axes and the height mode was used for the analysis. Dozens of parallel samples were examined for each specimen in order to obtain reliable statistical results. ANOVAs of the heights of the chains and particles were conducted to investigate the effects of heating mode and starch kinds on the nanostructures. Duncan's multiple range test was performed using SAS (Version 9.0, Statistical Analysis Systems, Cary, NC, USA). The heights of the chains and particles were recorded as means ± standard deviation. Comparisons that yielded *P*-values <0.05 were considered significant.

3. Results and discussion

3.1. Networks of microwaved potato starches

The effect of microwave heating on gelatinization of potato starches was compared with that of convective heating. Fig. 1a showed that the starch formed a membrane on mica when gelatinized by convective method. The thickness of the membrane was about 6.11 nm (Fig. 1b and c), detected by nanoindentation method (Arnault, Mosser, Zamfirescu, & Pelletier, 2002; Ruan & Bhushan, 1993). The membrane is thick and very large and hence we could not find the edge until in a large scan size, e.g. 80 μm × 80 μm. Potato starch formed networks under microwave heating (Fig. 2). Some molecules formed lamellas with holes interspersing among them. The depth of the hole was about 2.3 nm. Other molecules aggregated and connected with each other to form networks. Many nanoparticles scattered on the membrane (Fig. 2a, arrow A), with the apparent height of about 3.08 ± 0.61 nm ($n = 73$). These particles were not easy to define and did not participate in forming networks. The height of the formed networks ranged from 0.3 to 11 nm. Compared to the convective heating method, the uneven height of the networks caused by microwave heating suggested that it was incomplete gelatinization. If water absorption and swelling proceed equally, the starch chains should extend completely so as to form a uniform membrane.

3.2. Cap structure of microwaved potato starch chains

Chains are important characteristics of starch molecules, which are fundamental for understanding the starch structures and the reaction mechanisms at molecular level. It is well known that starch is composed of two polymers, amylose and amylopectin. Amylose is the constituent of starch in which anhydroglucose units are linked by α-D-1,4-glucosidic bonds to form linear chains. Former researchers (Giardina et al., 2001; Gunning et al., 2003; Morris, Gunning, Faulds, Williamson, & Svensson, 2005) observed the linear structures of amylose by AFM, the apparent

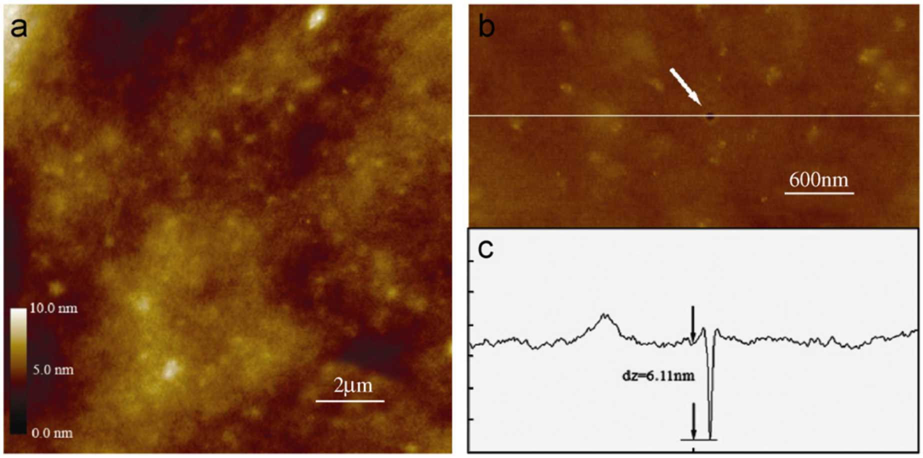

Fig. 1. AFM height image of potato starch gelatinized by convective heating method. (a) Scan size: 10 μm × 10 μm, height scale: 10 nm. (b) Section analysis of the thickness of the starch membrane, the hole as the arrow pointed was made by AFM indentation. Scan size: 3 μm × 1.5 μm, height scale: 10 nm. (c) The section analysis of the depth of deposited potato starches. The arrows show the depth of the hole (arrow pointed in part (b)) made by nanoindentation method.

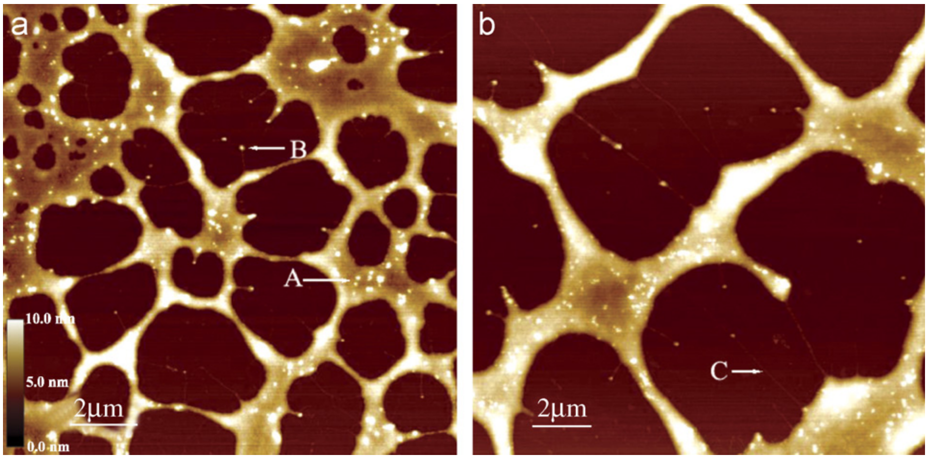

Fig. 2. Networks of potato starches gelatinized by microwave heating method. Parts (a) and (b) are two selected areas showing the uneven heating by microwave. Arrow A points to the randomly scattered nanoparticles; arrow B points to the cap ahead of the chain; and arrow C points to the rod-like chain. Scan size: 10 μm × 10 μm, height scale: 10 nm.

heights of the chains were 0.6–0.8 nm on molecular level. Amylopectin is the other constituent of starch linked mainly by α-1,4 linkages but with a greater proportion of α-1,6 linkages. However, the direct evidence of branched chain structures of amylopectin has not been demonstrated.

The effects of microwave heating on starch chain level could be studied by AFM. Liu et al. (2001) showed the pearl-like starch chains in AFM studies of microwave-assisted modification of starch. However, we observed linear chains of microwaved potato starch (Fig. 2b). Chains could be sorted into three kinds by the measured height (as shown in Table 1). The middle chains with the apparent height of 0.6 nm were similar to the previous reports (Gunning et al., 2003). The AFM image clearly showed many thicker rod-like chains with an equal height about

Table 1
Comparison of the height of chains or particles of potato and corn starch between convective heating and microwave heating

	Height of convective heating (nm)		Height of microwave heating (nm)	
	Potato starch	Corn starch	Potato starch	Corn starch
Thin chains	–	0.30 ± 0.07	0.31 ± 0.08	0.27 ± 0.06
Middle chains	–	0.61 ± 0.10	0.64 ± 0.06	0.58 ± 0.06
Rod-like chains	–	0.97 ± 0.09	1.02 ± 0.09	1.09 ± 0.09
Particles	2.86 ± 0.24	5.43 ± 0.84	3.08 ± 0.61	5.65 ± 1.19
Caps ahead of chain	–	–	5.62 ± 0.97	10.58 ± 4.38

Value in the same line with same superscript letters indicate no significant differences by the Duncan's multiple range test ($P < 0.05$).

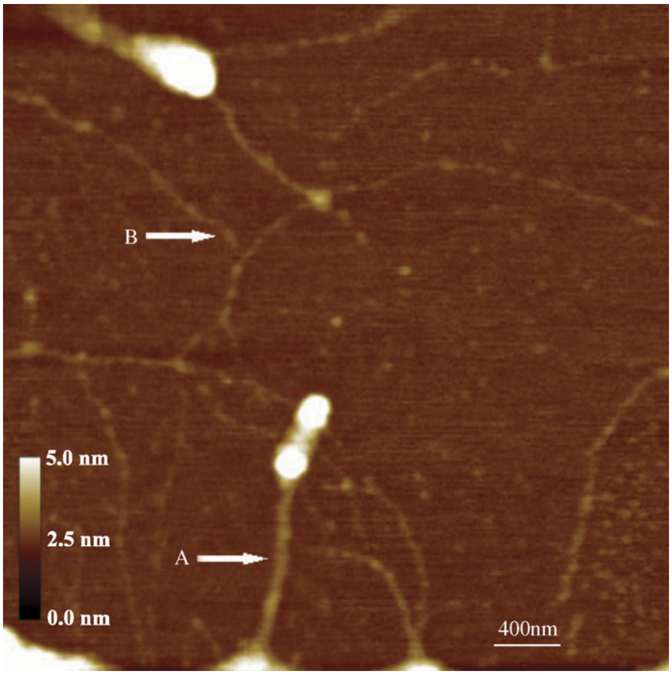

Fig. 3. A typical AFM height image of starch chains chosen from one part of Fig. 2a. Arrow A points to the rod-like chains, while arrow B points to the thinner chains. Scan size: 2 μm × 2 μm, height scale: 5 nm.

1 nm, which were comparable with V–H helical amylose (Fig. 2b, arrow C; Fig. 3, arrow A) (Imberty, Chanzy, Perez, Buleon, & Tran, 1988; Immel & Lichtenthaler, 2000). In addition, the AFM image also showed a few thinner chains with a measurable height of about 0.3 nm (Fig. 3, arrow B). These chains were postulated to be glucose monochains other than helical structures. Many branches were also found linked to them. The apparent height of chains was vertical distance shown in AFM images, which may be smaller than its real value due to the tapping force from AFM tips. The apparent width of the middle chains was measured as 23.54 ± 2.13 nm, which was much larger than its diameter because of tip-broadening effect (Liu et al., 2001). More importantly, particles ahead of potato starch chains—we called them as caps—were found after microwave heating in our cases (Fig. 2a, arrow B). The cap featured most of the imaged chains, and the height of the cap was about 5.62 ± 0.97 nm ($n = 47$). Some particles joined the chains linking the starch patches. Before drawing conclusion, it should be made clear what the caps are. First of all, through the analysis of the apparent height, they are much larger than chains. The caps could not be glucose monochains. Next, it was known that starch gelatinization is a process involved in the irreversible swelling by water uptake. As temperature increased, soluble component began to dissociate and diffused out of starch granules. The displayed chains in AFM images should be soluble components while the caps might be insoluble components. More than that, the uneven effect of microwave heating led to non-uniform swelling of starch. Therefore, it is reasonable to believe that the caps are clusters of glucan chains, which were not extended well and presented a particle. Such effect of microwave on potato starch may affect its properties, e.g. viscocities, and ultimately its application.

3.3. Networks of convectively heated corn starches

In contrast to potato starches, the effect of convective heating on corn starches was quite different. Fig. 4 shows the networks under convective heating. Corn starch chains linked to each other and deposited extensively on mica surface. The results were consistent with those of Liu et al. (2001). The height of the chains ranged from 0.23 to 1.19 nm. The scattered nanoparticles with height of about 5 nm were also presented (Fig. 4, arrows pointed). The topography of gelatinized corn starches was different from that of potato starches, which may be due to the different crystalline and organization modes between the two kinds of starches.

3.4. Cap structures of microwaved corn starch chains

Microwave heating had a different effect on corn starch compared to that from convective heating. AFM images showed that the starch chains were capped with a nanoparticle (Fig. 5a, arrow A) (the height value shown in Table 1). As mentioned above, the chains might be soluble components dissociated from starch granule. The results were similar to those of potato starches, except the height of the caps which was from 6.5 to 20 nm. The height of rod-like chains was 1.09 ± 0.09 nm. We also found pearl chains similar to the structures reported by Liu et al. (2005)

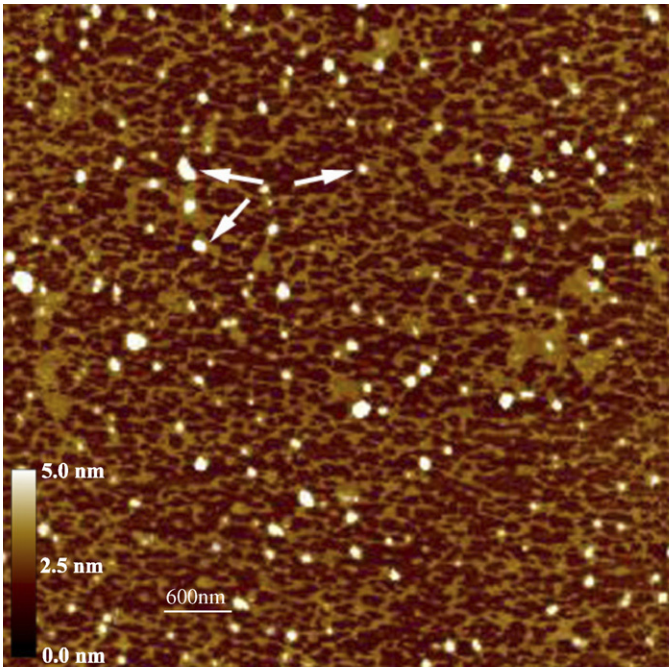

Fig. 4. AFM height image of corn starch networks gelatinized by convective heating method. (a) Scan size: 3 μm × 3 μm, height scale: 5 nm.

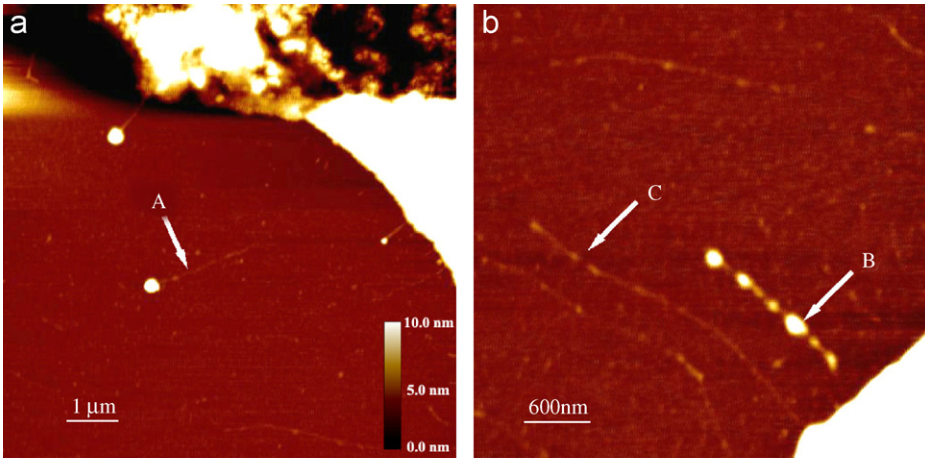

Fig. 5. AFM height images of microwaved corn starches. The bright areas were patches of corn starch granules. (a) Arrow A points to rod-like chains. Scan size: 5 µm × 5 µm, height scale: 10 nm. (b) Arrow B points to pearl chain structures and arrow C points to thinner chains. Scan size: 3 µm × 3 µm, height scale: 10 nm. The white masses were high parts of the image, which may be incomplete gelatinized parts.

(Fig. 5b, arrow B). In addition, AFM images also revealed thinner chains with the height of about 0.3 nm (Fig. 5b, arrow C). No obvious networks were found in corn starch when heated by microwave.

Potato starches were B-type crystalline structures, while corn starches were A-type crystalline structures. The different inside regulation affects their physicochemical properties and application. Table 1 shows the different effects of microwave heating and convective heating on starch gelatinization between potato starch and corn starch. The similarity was that both starches presented features of capped chains under microwave heating, and each of them showed three kinds of chain with differences in diameter. Potato starches showed networks while corn starch did not, which indicated that the swelling of starch depended on the inner texture of granules. Potato starches showed membrane instead of chain structures or networks under convective heating. The AFM data suggested potato starch to possess stronger paste viscosity than corn starch. The networks or helices may affect water retention, so do they as to the appearance and texture of microwaved starch-based food.

Although the results are repeatable and reliable, we could not say that the cap features and the pearl chains in AFM images were not artifacts of drying. We draw conclusions dependent on the fact that controls and repeats performed very well. The fact that potato starches formed networks and both starches formed the capped structures under microwave heating indicated incomplete gelatinization. It was reasonable to think that incomplete gelatinization led to decrease in the viscosity of paste. Providing DSC measurement and Brabender viscosity properties during microwave heating gains importances. However, the two methods mentioned above are not easy to fix with microwave sets. It should be noted that both measurements need solid native starch, while after microwave heating, the starches became paste.

The changes in starch texture during microwave radiation are of crucial concern for making starch-based food. The forming of networks of starch under microwave heating could enhance the water-holding capability. Structural information disclosed by AFM imply that starches were incompletely gelatinized through rapid water transfer and non-uniform heating of the microwave. Steps should be taken to overcome the noted shortcomings of microwave heating is attempted to make crisp crust and fresh-like food. The results in this paper are useful in the choice of starch for microwave process, such as coat potato starch on crust to prevent shrinking. Herein, we simply studied two kinds of starches. Starches from other plant resources or with different amylose content should be considered in future.

4. Conclusion

This work demonstrates the effects of heating modes and the kind of starch on the nanostructure of starch molecules. AFM images showed that the uneven microwave heating caused potato starch to form networks. Both starches formed the capped chains structures under microwave heating. The data indicated microwave heating caused incomplete gelatinization of starches. Heating modes influence the potato starch much more compared to its influence on corn starch. The nanostructure information would benefit is attempts at selecting materials microwaved-food development.

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