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Image study of pectin extraction from orange skin assisted by microwave

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Abstract

The process of pectin extraction from orange skin assisted by microwave has been researched by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The effects of microwave radiation on organization of the orange skin and the changes on this orange skin organization during the extraction have both been analyzed.
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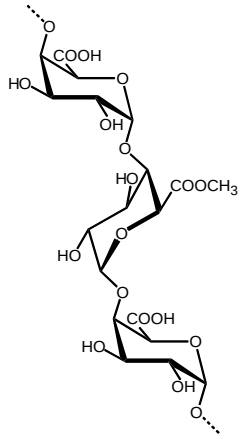
Keywords: Microwave; Pectin; Extraction; SEM; AFM

1. Introduction

Pectin is the structural unit of fresh cells and the junction between the cells in advanced land plants. It mostly exists between the cell walls and its function is to agglutinate the cells to form a compact junction. Some pectin also exists in the cytosol (Wu, 1987). The structure of pectin is illustrated as follows:



A variety of methods of production of pectin have already been reported by many researches (Liu Zhiwei, Wen Nan, & Zheng Mengyu, 2002; Kovacs, 1998; Douglass, Fitzgerald, Ingebrethsen, & Tyson, 2004). The process of the traditional method includes degradation by acid and deposition (Zhang & Liu, 1997), using acid solution at pH 2.0 and a temperature of 80–100 °C. The process lasts more than 1 h. These conditions lead to protein degradation, so this traditional method is not good for either quantity or quality of the pectin extraction. Therefore, it is necessary to establish a new method, by which the pectin could be extracted in a shorter time and in better quality. This paper focuses on the extraction of pectin from the organization of the orange skin under microwave condition, and scanning electron microscopy (SEM) and atomic force microscopy (AFM) are used to analysis this process (Huang, 1982).

2. Materials and methods

2.1. Materials and agents

Oranges (Qi Orange) were purchased from the Star Supermarket on the Tongbai Road in Zhengzhou, in September 2004.

Aqueous ethyl alcohol 95% (v/v), absolute ethyl alcohol, aqueous glutaraldehyde 50% (v/v), osmic acid, and isoamyl acetate were all analytical grade reagents.

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2.2. Instruments

Microwave oven: ER-761MD, Haier of Qingdao, China; SEM model: Hitachi Ltd 5-570, Japan; NanoScope IIIa SPM: Digital Instruments\Veeco Inc., Santa Barbara, CA, USA.

2.3. Methods

2.3.1. Extraction of the pectin from orange skin under the microwave method

Sliced orange skin (200 g) was put into 0.3% (w/v) sodium bisulfate solution (1000 mL). After 30 min it was washed with clean water till the washing water was colorless. After drying, the skin was dipped into water (1000 mL) and the pH adjusted to 2 with 0.5 M HCl solution. The samples were kept stable for 10 min. Then the samples (including the liquid) were put in the center of the microwave oven, and heated at 85 °C (the microwave oven in this experiment has the function of controlling the temperature) for 5 min. The next step of this process was to filter the hot mixture through a four-layer-gauze, and then cool it to room temperature. Subsequently, saturated aqueous $Al_2(SO_4)_3$ solution (50 mL) was added slowly into the filtrate and the pH was changed to 4 by the addition of ammonia solution. After these treatments, the filtrate was stirred for 0.5 h, and then held still for 1 h to allow the pectin–aluminium hydroxide gel to form; the gel was then collected by centrifugation. The pectin–aluminium hydroxide gel was then washed with clean water and then dried at room temperature. After being treated with acidified ethyl alcohol (60% ethyl alcohol/conc. HCl; 5:1 v/v), the gel was filtered by a sand core filter, and washed twice with neutral 60% ethyl alcohol, then dehydrated by steeping in absolute ethyl alcohol (1000 mL), and finally, air dried at 50 °C.

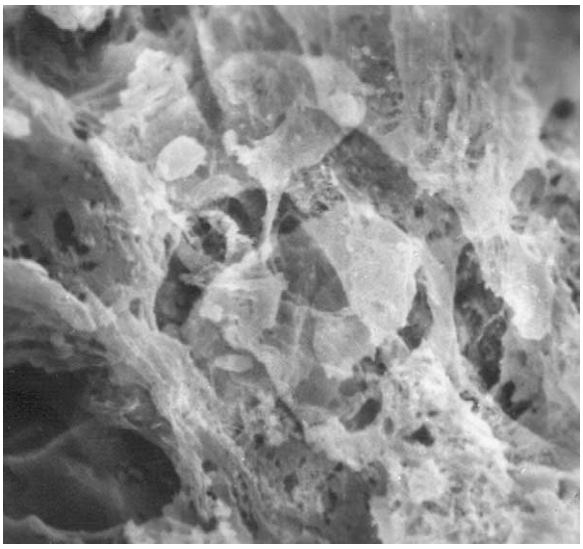

Fig. 2. SEM scan at 25 kV of the structure of the cells of orange skin organization extracted by traditional method for 12 h, X2000, original width of picture was 25 μm.

2.3.2. Preparation of the samples for examination by SEM and AFM

Both the two kinds of pectin samples that were extracted by microwave method and traditional method (Zhang & Liu, 1997), respectively, and the orange skin that had been dried naturally were used for scanning by SEM and AFM (Huang, 1982).

The preparation of orange skin samples for SEM: (1) selecting orange skin samples and slicing them; (2) treating the orange skin sample by fixation, dehydration, drying and gold plating; (3) observing the orange skin samples by SEM.

The preparation of pectin samples for SEM: (1) daubing the pectin samples on the coppery plate; (2) plating gold on

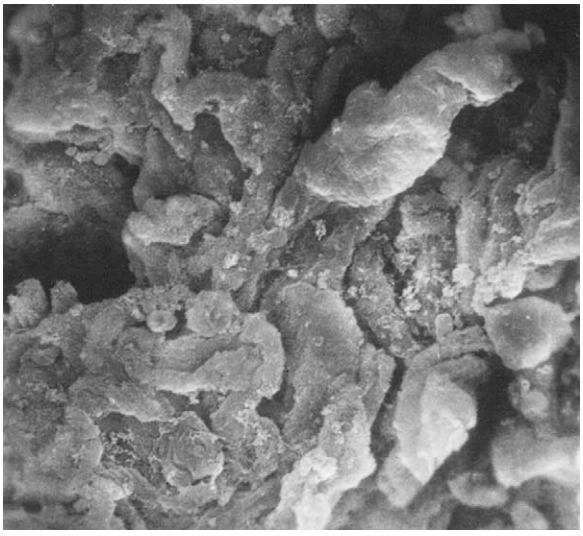

Fig. 1. SEM scan at 25 kV of the structure of the orange skin organization which had been dried naturally, not treated, X700, original width of picture was 43 μm.

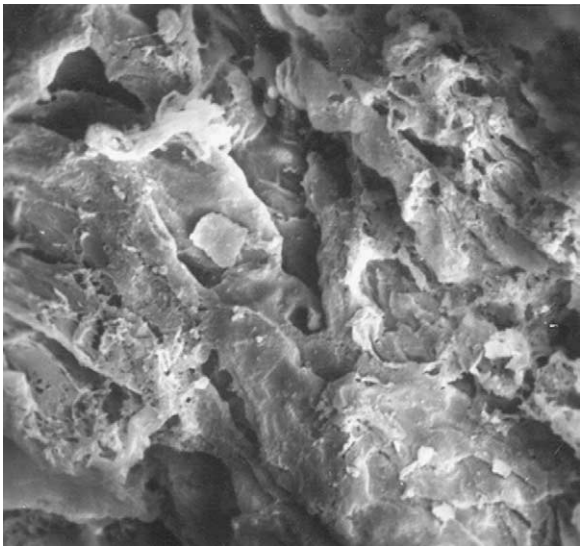

Fig. 3. SEM scan at 25 kV of the structure of cells of orange skin organization extracted by traditional method for 18 h, X2490, original width of picture was 12 μm.

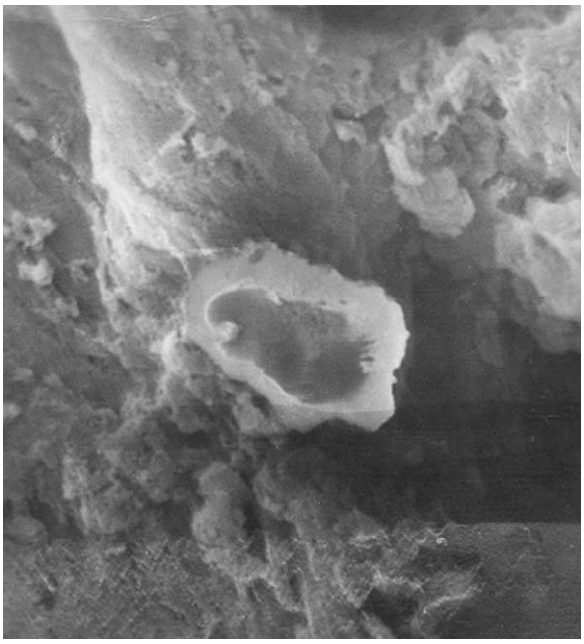

Fig. 4. SEM scan at 25 kV of the structure of cells of orange skin organization by microwave heating for 5 min, X2490, original width of picture was 12 μm.

the pectin samples in the vacuum; (3) observing the pectin samples by SEM.

The preparation of pectin samples for AFM: (1) dissolving the pectin samples absolutely in water by heating; (2) dropping the pectin samples on the cleavage plane of mica; (3) fixing the pectin sample by absolute ethyl alcohol; (4) observing the pectin samples by AFM.

3. Results and discussion

3.1. The structural changes of orange skin organization in the two kinds of extracting processes

The structural changes of orange skin's organization in the extracting process, respectively, according to the microwave

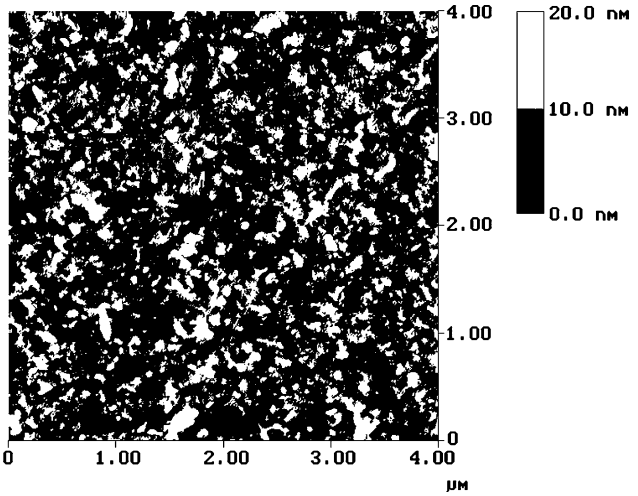

Fig. 5. AFM scan, at 220 V, of the structure of pectin extracted by microwave heating method for 5 min, original width of picture was 4.0 μm.

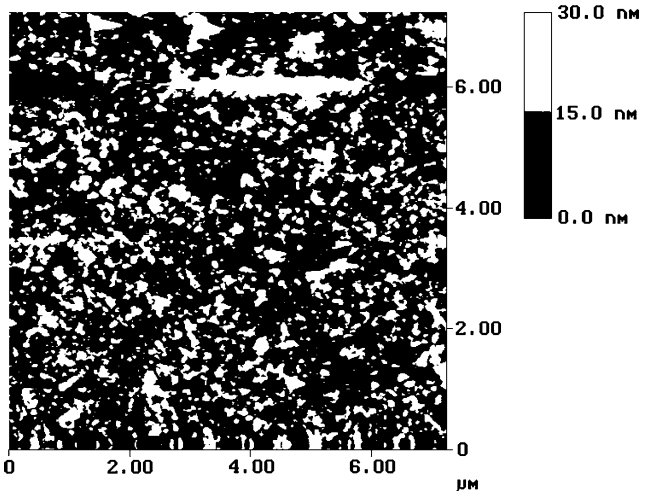

Fig. 6. AFM scan at 220 V of the structure of pectin extracted by microwave heating method for 5 min, original width of picture was 6.0 μm.

heating method and the traditional extracting method as observed by SEM and AFM are shown in Figs. 1–10.

Fig. 1 shows that the structure of the organization of the orange skin just dried naturally is generally still complete and compact. The same organization has begun to disintegrate under the traditional extraction method for 12 h, and some interstices begin to appear (see Fig. 2). The orange skin organization has more completely disintegrated after 18 h, and the interstices have been very clear and common (see Fig. 3). However, the degree of disintegration under the traditional method for 18 h is less intense than that under the microwave heating method for 5 min (see Fig. 4). Fig. 4 shows that the degree of disintegration is so sharp that the cell of the orange

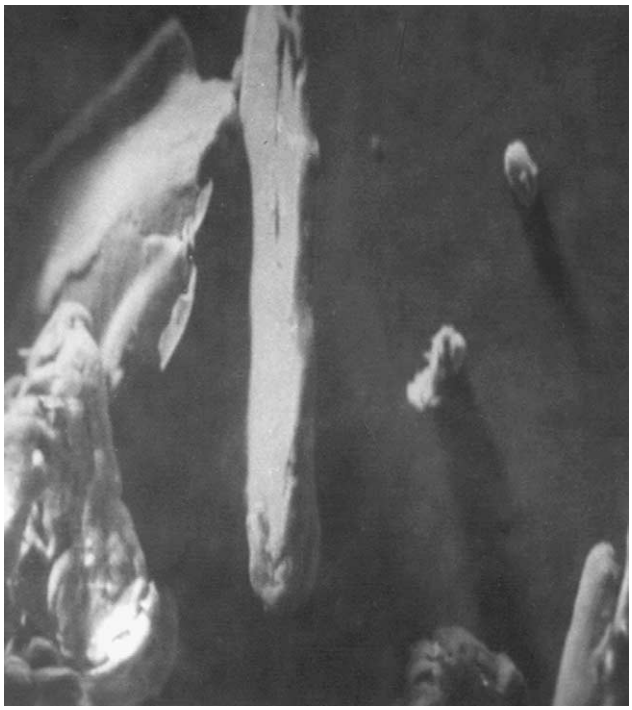

Fig. 7. SEM scan at 25 kV of the pectin extracted by traditional heating method, X150, original width of picture was 200 μm.

Fig. 8. SEM scan at 25 kV of the pectin extracted by traditional heating method, X150, original width of picture was 200 μm. This figure and Fig. 7 are under the same conditions, but they describe the difference from the different angle and side.

skin organization is split, and the structure becomes looser than that in Figs. 1–3. Since the ratio of pectin extraction is related to the amount and shape of the interstices in the samples, the efficiency of extraction under the microwave heating method is inevitable higher than that under the traditional method. Figs. 5 and 6 show the structure of the pectin that was extracted by

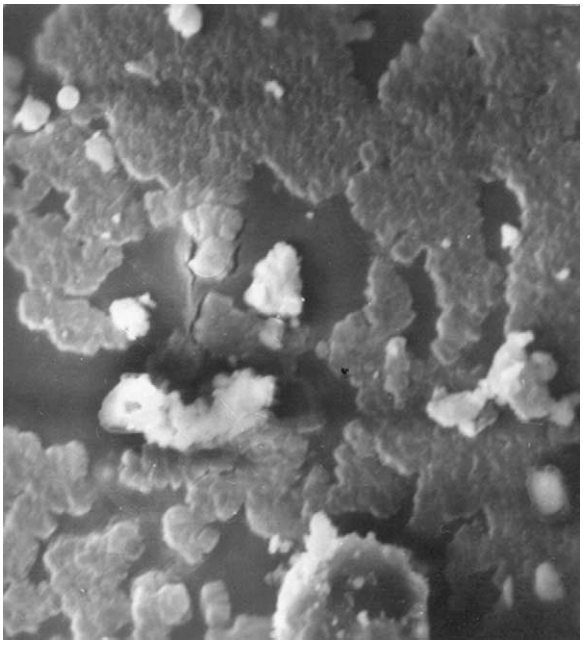

Fig. 9. SEM scan at 25 kV of the pectin extracted by traditional heating method, X2000, original width of picture was 25 μm.

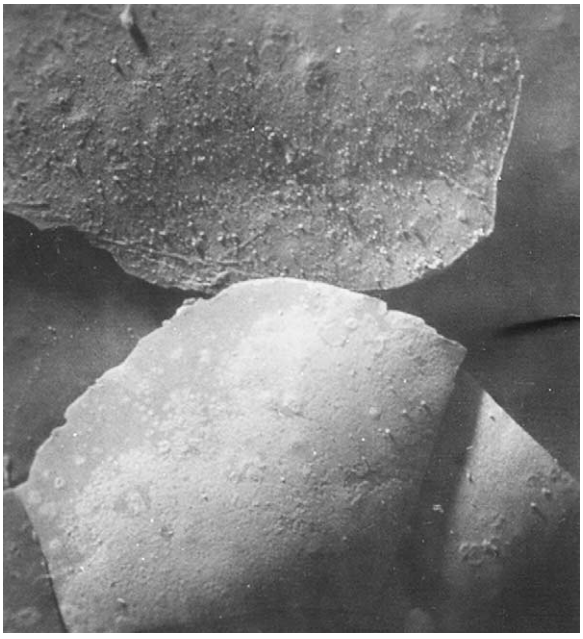

Fig. 10. SEM scan at 25 kV of the pectin extracted by traditional heating method, X2900, original width of picture was 100 μm.

the microwave heating method as scanned by the AFM. This scan refers to the nano-structure of the pectin. The results (see Figs. 5 and 6) indicate that the two kinds of pectin construction in nano-structure are all clubbed. As shown in Figs. 5 and 6, the length of the high methoxyl content group is longer than that of low methoxyl content group in nano-club. In the structure of pectin, the basic unit is D-galacturonic acid. Its conglomeration is not apt to crystallize. With AFM, we can observe configurations in big molecules, all of which have different 'long chains' (Ridley, O'Neill, & Mohnen, 2001).

The results indicate that microwave radiation has the stronger destructive effect on the structure of orange skin organization, and the swelling effect of the microwave even forces of the cells to split (see Fig. 4).

Scan of pectin samples extracted using the traditional method and the microwave heating method

The structure of the pectin samples which were extracted according to the microwave heating method and the traditional method were scanned by SEM. Figs. 7–10 show that the pectin formation according to different methods present different shapes. The swelling effect of the microwave was such that the cluster formation of the pectin was broken, and became particulate in a way that was similar to crystal. Meanwhile under the traditional method, the pectin mainly remained as the cluster formation.

4. Conclusions

1. It has been confirmed that the pectin release from orange skin using microwave conditions should be a rapid disintegration process. Having absorbed the energy of the microwave, the temperature of the cell of the orange skin will sharply increase in a very short time, and the pressure

in the cell will exceed the maximum, which the cell wall can endure, so the cell will split and the pectin and other inclusion can release itself. As a result, the skin tissues rapidly and extensively are opened up by the microwaves.
2. The time required for the extracting process is reduced from the 1 h of the classical methods to 5 min by the microwave method by virtue of the microwave disintegration processing.
3. It has been confirmed that there is a swelling effect on the cells of the orange skin under microwave radiations of 2450 MH, 1000 W.
4. Although microwave radiation with 2450 MH, 1000 W is used in this experiment, other microwave radiations with different wavelengths are usable.
5. There are many plant natural materials which can be expected to yield carbohydrate polymers through expedited liberation extraction processes facilitated by microwave disintegration.

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