Acta Botanica Sinica
植 物 学 报 2004, **46** (10): 1249-1255 http://www.chineseplantscience.com

Quantitative Roughness Analysis of Post-harvest *Agaricus bisporus* by Atomic Force Microscopy

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Abstract: The moisture loss degree is important in determining the quality of post-harvest mushroom (*Agaricus bisporus* (Lange) Sing). Quantitative roughness analyzed by atomic force microscopy (AFM) was proposed to denote the degree of shrinkage, with arithmetic average roughness (R_a) and root mean square roughness (R_q) as parameters. The initial value of R_a was (30.035±1.839) nm, while those of 2 ℃, 25 ℃ and dynamic temperature on the 2nd day were (40.139±3.359) nm, (54.393±13.534) nm and (41.197±6.555) nm, respectively. There is a similar tendency for the results of R_a and R_q. Both values of roughness increased in duration of storage and with increasing temperatures. The three-dimensional profile of the pileus epicutis could signify the process of water evaporation intuitionally. The tendency was in accordance with the roughness results, especially for the earlier stage of the storage (0-2 d). The outcome of roughness analysis could signify the differences of storage conditions. It was shown that the roughness measured by atomic force microscopy effectively reflected the moisture loss degree of the mushroom pileus epicutis during post-harvest storage.

Key words: mushroom; roughness degree; atomic force microscopy (AFM); moisture loss; modified atmosphere

Appearance is a primary factor in quality judgment of fruits and vegetables (Veraverbeke *et al.*, 2003a). A loss in weight of about 5% often causes produce to lose freshness and appear wilted (Kang and Lee, 1998). High relative humidity (RH) could help minimize the shriveling, but too high RH will result in fungal decay during storage (Veraverbeke *et al.*, 2003b). It is inevitable that shriveling of produce happens.

The traditional criteria used to determine the degree of shriveling are sensory evaluation and weight loss. Weight loss is not uniform in different parts of the fruit body. However, consumers are concerned more about the appearance of the skin of produce.

Instrumental measurements are often preferred to sensory evaluations in research and commercial situations because they reduce variances in judgment among individuals and can provide a common language.

Most recently emphasis has been placed on developing sensors for real-time, non-destructive sorting (Abott, 1999). Neither light microscopy (LM) nor scanning electron microscopy (SEM) produces qualitative topographical data in a straightforward manner (Wan and Tian, 2002; Wang and Huang, 2003). The structures of the different parts of the mushroom coatings were studied by SEM but without the roughness data of the coatings (Hershko and Nussinovitch, 1998a). Veraverbeke *et al.* (2003a) investigated fruit surface layers with confocal laser scanning microscopy (CLSM) and environmental scanning electron microscopy (ESEM). Staining was needed and CLSM was not sufficient because this technique only detects fluorescence and reflection results in a much blurred image (Liu *et al.*, 2003).

Gibbs and Bishop (1996) proposed using a geostatistical technique to describe bio-film surface roughness. Height measurements are accurate to 1 μm or less, which is a low resolution for investigating the changes of roughness. Burdon and Clark (2001) had monitored the impact of water loss using serial quantitative proton magnetic resonance imaging; however, these results were not intuitional.

The way in advance of biology and material field on nanometer scale in the past decades has been found in application with atomic force microscopy (AFM). The advantage of high resolution allows precise topography imaging in a controlled way and Pico Newton magnitude force

Received 30 Mar. 2004 Accepted 6 Jul. 2004
Supported by the Key Programs for Science and Technology Development of Shanghai Science Committee (023912063).
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measurement. AFM could image kinds of samples, such as unstained and uncoated structures, in air or fluid, permitting direct observation of native specimens and ongoing processes under native or near-native conditions. The topology of plant materials has been studied by AFM (Wilbert *et al.*, 1998; Round *et al.*, 2000). Hershko and Nussinovitch (1998b) compared the roughness between the onion skin surface and the chloroform-cleaned onion surface. Also, there are some reports about qualitative roughness analysis with AFM for plastic films and metal surfaces (Darrot *et al.*, 1995; Reed *et al.*, 1998; Lindseth and Bardal, 1999). However, to our best knowledge, there is no report about the changes of the roughness of produce during storage.

The main objective of this paper is to propose that the roughness of mushroom pileus epicutis could be used as one of the evaluating criteria for signifying the appearance during post-harvest. AFM was applied to make quantitative and accurate measurements of the topography of the mushroom surfaces.

1 Materials and Methods

1.1 Materials

Mushrooms (*Agaricus bisporus* (Lange) Sing), 4 h after harvested, were bought from Qibao supermarket in Shanghai, then pre-cooled at 2 ℃ for 12 h. After being sorted out and wiped off the stalk, mushrooms were washed and dipped in 0.1 mol/L NaCl to inactivate the polyphenol oxidase. Then mushrooms were drained (2 000 Pa, 20 min) by the vacuum evaporation machine (Shanghai Yiheng Science and Technology Co. Ltd.) to remove water on the surfaces and packed in 0.035 mm low density polyethylene (LDPE) packages, and then divided into three groups and from now on the time was recorded as 0 d. Groups 1 and 2 were stored at 2 ℃ and 25 ℃, respectively, through all the test. However, group 3 was stored at different temperatures for different stages to simulate the cold chain in commercial distribution, which is shown below:

25 ℃, 2 h → 2 ℃, 6 h → 15 ℃, 0.5 h → 4 ℃, 72 h → 15 ℃, 0.5 h → 2 ℃, 5 h → 25 ℃, 0.5 h → 2 ℃, 48 h → 25 ℃

1.2 Atomic force microscopy (AFM) measurement and roughness analysis

Samples were cut into thin pieces which fit into AFM imaging, and were stuck onto mica surface by double-sided tape, mounted onto the sample stage, then scanned with Tapping mode at the scan speed of about 1-2 Hz.

Tapping mode was carried out using a multimode NanoScope IIIa AFM (digital instruments Santa Barbara, CA, USA) equipped with a Si_3N_4 cantilevered scanner with a 12 μm × 12 μm scan size and a 4-μm vertical range. It has a high resolution with about 0.1 nm for vertical range and 1-2 nm for lateral (Darrot *et al.*, 1995).

To make the results comparable, the images were obtained from the center area of each surface. Since the area was much small, it did not contain any vasculature. Before imaging each sample, the integrity of the AFM tip was verified by imaging a reference standard with a known roughness of 5-7 nm (Reed *et al.*, 1998).

Atomic force microscopes are generally limited to small scanned areas. Three images of different zones were examined and offline analyzed with version 5.12 software on each specimen in order to average the roughness value (Darrot *et al.*, 1995).

Height measurements were performed on small (from 2.0 μm × 2.0 μm to 5.0 μm × 5.0 μm) surfaces of mushroom pileus epicutis after different storage conditions. Only selected representative images of each type are presented in this paper.

The height variation is represented by a color scale in which pink is high and purple is low for all images. Different scales are used in the vertical and horizontal scale.

Two amplitude parameters have been used. The arithmetic average roughness, R_a, and the root mean square (RMS) roughness, R_q, are given by:

$$R_a = \frac{1}{n_x n_y} \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} \left| Z(i,j) - Z_{\text{ave}} \right| \quad (1)$$

$$R_q = \sqrt{\frac{\sum_{i=1}^{n_x} \sum_{j=1}^{n_y} [Z(i,j) - Z_{\text{ave}}]^2}{n_x n_y}} \quad (2)$$

Where $Z(i, j)$ denotes the topography data for the surface after specimen tilt-correction, Z_{ave} is the average surface height, i and j correspond to pixels in the x and the y directions, and the maximum number of pixels in the two directions are given by n_x and n_y.

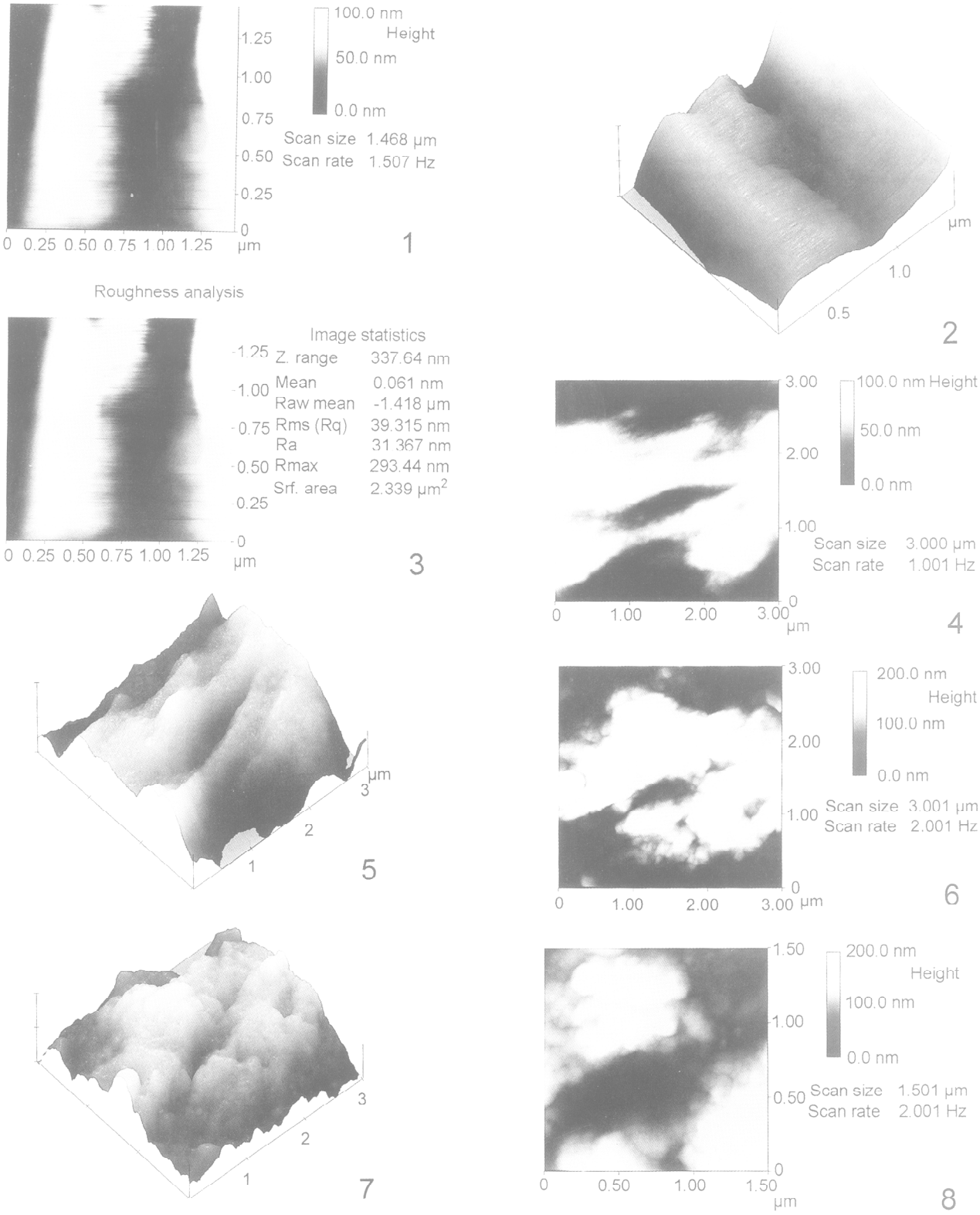
All parameters used are well known from surface metrology. The calculations were made using tilt-corrected topography data (Lindseth and Bardal, 1999).

1.3 Statistical analysis

Statistical analysis of results through ANOVA ($P < 0.05$) and Duncan's multiple range test were performed using SAS 8.0.

2 Results

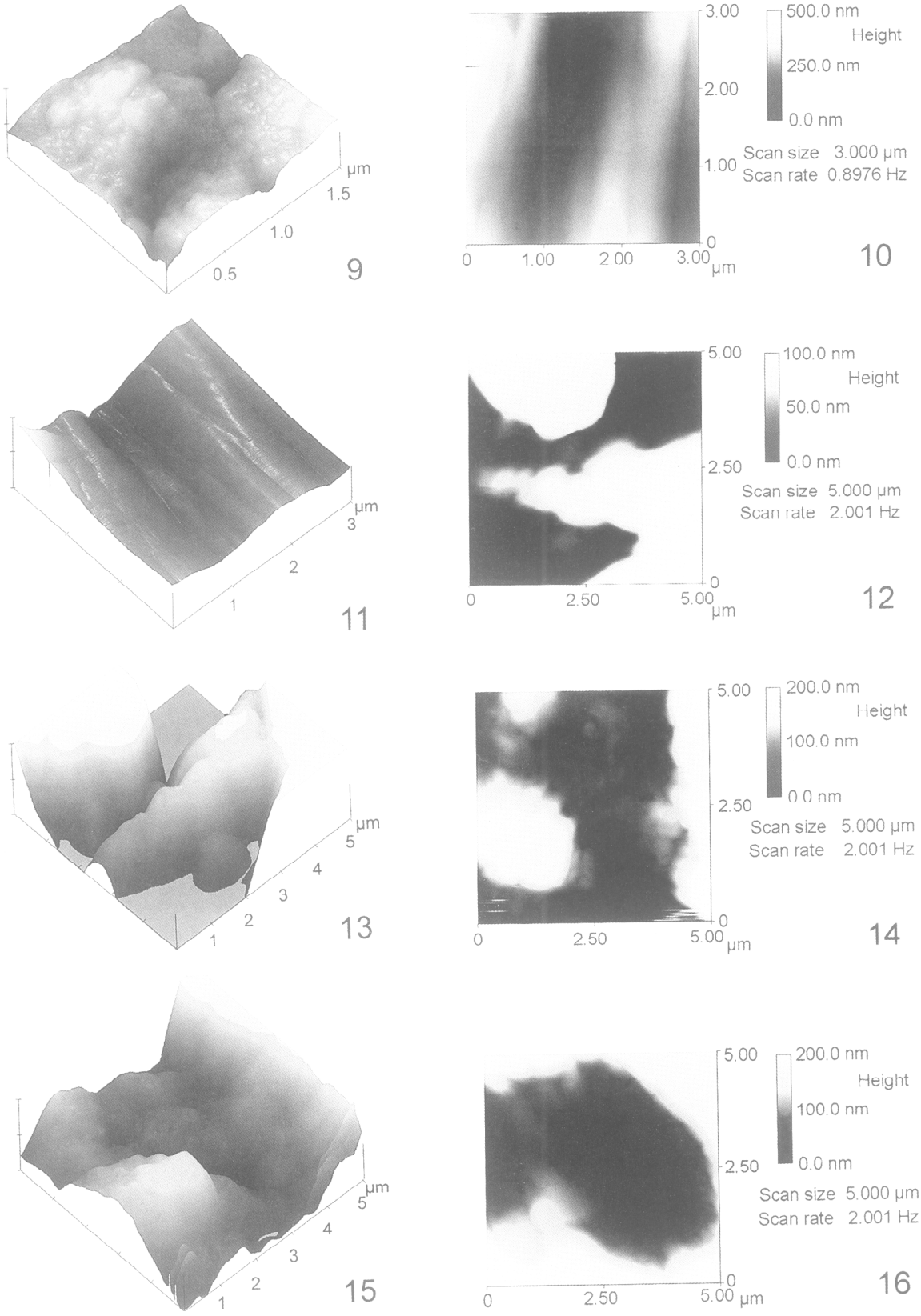
Figures 1 and 2 show the plane and three-dimensional profiles of the mushroom pileus epicutis before being divided into three groups, respectively. The R_a was 31.367 nm (shown in Fig.3), other two parallel samples were 30.801



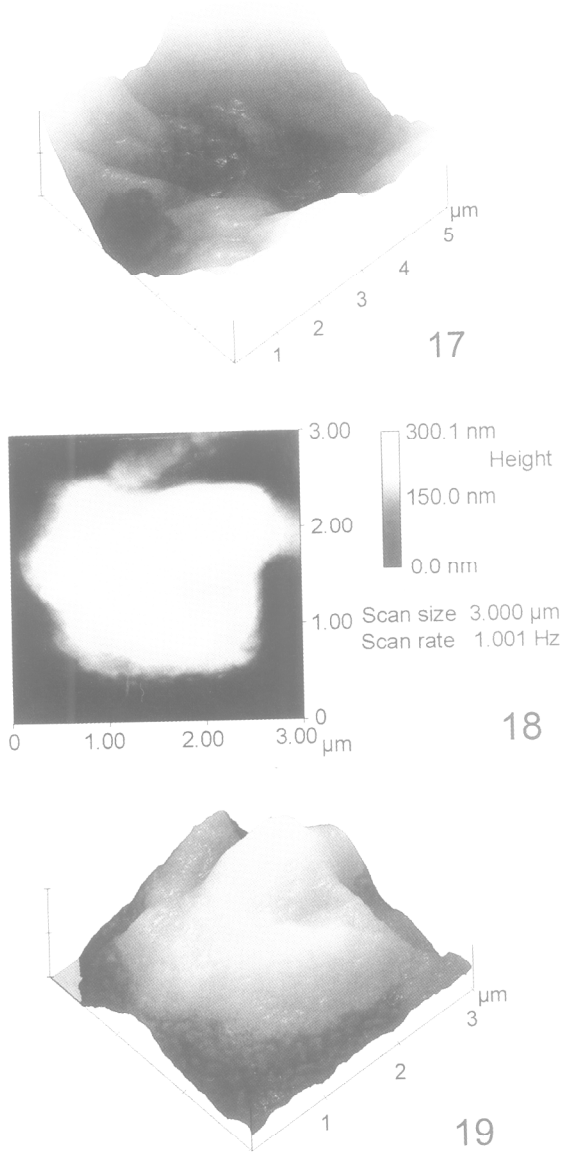
Figs.1-8. Plane and three-dimensional (3-D) profiles of mushroom pileus epicutis at different storage stages by atomic force microscopy (AFM). **1.** Plane, 0 d. **2.** 3-D, 0 d. **3.** Roughness analysis of the pileus epicutis at 0 d. **4.** Plane, 2 **℃**, 2 d. **5.** 3-D, 2 **℃**, 2 d. **6.** Plane, 25 **℃**, 2 d. **7.** Plane, 25 **℃**, 2 d. **8.** Plane, dynamic temperature, 2 d.

nm and 27.937 nm (not shown in the paper). And the statistical result was (34.033 ± 5.116) nm (Table 1).

Figures 4-9 show the plane and three-dimensional profiles of mushroom pileus epicutis of group 1 to group 3



Figs.9-16. Plane and three-dimensional (3-D) profiles of mushroom pileus epicutis at different storage stages by atomic force microscopy (AFM). **9.** 3-D, dynamic temperature, 2 d. **10.** Plane, 2 **℃**, 4 d. **11.** 3-D, 2 **℃**, 4 d. **12.** Plane, 25 **℃**, 4 d. **13.** 3-D, 25 **℃**, 4 d. **14.** Plane, dynamic temperature, 4 d. **15.** Plane, dynamic temperature, 4 d. **16.** Plane, 2 **℃**, 6 d.



Figs.17-19. Plane and three-dimensional (3-D) profiles of mushroom pileus epicutis at different storage stages by atomic force microscopy (AFM). **17.** Plane, 2 **℃**, 6 d. **18.** Plane, dynamic temperature, 6 d. **19.** Plane, dynamic temperature, 6 d.

after two-day storage, and Figs.10-15 show the plane and three-dimensional profiles after four-day storage, and Figs. 16 and 19 show the plane and three-dimensional profiles of groups 1 and 3 after six-day storage. The group 2 was not assayed on the 6th day storage because the samples had rotted.

Table 1 shows the statistical results of R_a for all the groups. And Table 2 shows R_q after the similar process for these groups.

3 Discussion

It was clear from Table 1 that the roughness was different with different storage conditions and the roughness values of group 1 to group 3 increased with the time. Figures 1-19 show the intuitional process of the moisture evaporation from mushroom pileus epicutis. For example, the three-dimensional profile of the mushroom pileus epicutis before storage was much smooth (shown in Fig.2), but fluctuated after two-day storage (from Fig.4 to Fig.9). The higher the temperature was, the more rough in profile. This tendency was similar from the 2nd to the 6th day storage. However, the trend from the beginning to the 2nd day was more obvious than that from the 2nd to the 6th day, which was a great help to know the quality of mushroom after post-harvest (Hershko *et al.*, 1998). R_a and R_q have the similar tendency for these groups.

For preserving the integrity of samples, the tapping mode, which was developed specifically for soft materials, was used. With this technique, the probe oscillated vertically so that it could lightly tap the sample during imaging rather than slide over the surface, which virtually eliminated lateral shearing and sample damage for the majority of specimens and improved the lateral resolution of the AFM image (Yang *et al.*, 2003).

Hershko and Nussinovitch (1998a) had reported that the R_a of fresh and dry mushroom were (2.1 ± 0.8) μm and (2.2 ± 0.5) μm, respectively. The values are larger than in our experiments. However, the maximum height of onion skin is only 628 nm, which showed the maximum was near our experimental value. The scanned size was greatly smaller than Hershko and Nussinovitch (1998b) used. It is important to remark that the roughness value depends on the scanned area and on the number of data points; roughness decreases in line with the scanned area because of the surface statistics, i.e. the fractal behavior (Darrot *et al.*, 1995). AFM allows imaging at ambient conditions but is useful only for flat surfaces. Height differences of a specific surface exceeding the z-piezo range can cause damage of the tip, and steep or rough structures result in unrealistic images because of the limited aspect ratio of the tip (Fig.7). But it is an appropriate method for investigating the fine structures of individual areas. The wax platelet assayed 7-10 nm (Ensikat *et al.*, 2000). And unpolished stainless steel was (75 ± 29) nm of R_a (Verran *et al.*, 2000). Compared with these values, the results of mushroom in this paper were credible.

AFM are generally limited to small scanned areas. Images from these microscopes might be helpful when

Table 1 Effect of storage conditions on the arithmetic average roughness (R_a) of mushroom pileus epicutis/nm

Group	Storage time (d)			
	0	2	4	6
1	30.035±1.839 [a]	40.139±3.359 [a]	58.593±12.330 [b]	98.480±7.804 [b]
2	30.035±1.839 [a]	54.393±13.534 [a]	164.81±26.956 [a]	na
3	30.035±1.839 [a]	41.197±6.555 [a]	88.825±8.843 [b]	141.07±18.477 [a]

Values shown are mean ± *SD* where $n = 3$. Values in the same column with the same superscript letters (a or b) indicate no significant differences by the Duncan's multiple range test ($P < 0.05$). na, not analysis because of out of marketing value.

Table 2 Effect of storage conditions on the root mean square roughness (R_q) of mushroom pileus epicutis/nm

Group	Storage time (d)			
	0	2	4	6
1	36.656±3.475 [a]	49.308±4.549 [a]	75.055±17.038 [c]	123.613±14.888 [b]
2	36.656±3.475 [a]	68.581±17.489 [a]	200.51±15.123 [a]	na
3	36.656±3.475 [a]	50.213±7.800 [a]	116.327±10.015 [b]	171.213±24.311 [a]

Values shown are mean ± *SD* where $n = 3$. Values in the same column with the same superscript letters (a or b) indicate no significant differences by the Duncan's multiple range test ($P < 0.05$). na, not analysis because of out of marketing value.

evaluating topographic data, since these are well established methods for characterizing surfaces, and also contrast mechanism of LM and SEM are well known (Lindseth and Bardal, 1999). To appreciate the quality of homogeneity of the samples, on a large scale, it is also advised to use the SEM in combination with the AFM, each technique has its own pertinent scale of investigation and thus they prove very complementary (Darrot *et al.*, 1995).

The present work is not only important for characterization of mushroom surfaces. It should be relevant for any problem requiring topography measurements of strongly reflecting produce surfaces, having topographic features from around 10 μm and down well into the sub-micrometre range.

4 Conclusion

The roughness analysis gained from AFM was effective in determining the shrinkage degree of the mushroom during storage. Both values of R_a and R_q increased in duration of storage and with increasing temperatures. The three-dimensional profile of the pileus epicutis could intuitionally signify the process of water evaporation. And the surface would become rougher with the increment of time and rise of temperature. It is appropriate for R_a or R_q roughness measured by AFM to denote the appearance quality of mushroom, especially for the early stage of storage. It was shown that the roughness measured by AFM effectively signified the moisture loss of the mushroom pileus epicutis during post-harvest.

Acknowledgements: The authors would like to thank Dr. Ian B. Ferguson (The Horticulture and Food Research Institute, New Zealand) for his useful suggestions and Dr. LAN Yu-Bin (Fort Valley State University, USA) for his careful reading on this manuscript.

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(Managing editor: WANG Wei)