Visualization and quantitative roughness analysis of peach skin by atomic force microscopy under storage

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Abstract

Skin status of produce is important in determining fruit quality. Sensory evaluation is often used to express this characteristic. A new method, atomic force microscopy (AFM), was proposed to denote the changes of skin status. Arithmetic roughness (R_a) and root mean square roughness (R_q) of 'Jinxiu' yellow peach (*prunus persicu* L. Batsch.) were analysed by AFM. The R_a and R_q values increased with storage time in both controlled atmosphere (CA) and regular air (RA) storage, and the values of CA group were smaller than that of RA group. There is a linear correlation between R_a and R_q. The three-dimensional profiles of the skin could also be gained by AFM. The results indicate that the roughness values increase with the storage time, and the roughness of CA group increases slower than that of RA group.
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1. Introduction

Weight loss of about 5/100 g often causes fresh produce to lose freshness and appear wilted (Kang & Lee, 1998). High relative humidity (RH) could help minimize weight loss, but too high RH results in fungal decay (Veraverbeke, Verboven, Oostveldt, & Nicolai, 2003b). It is almost inevitable that the skin status of produce changes with storage time.

The traditional criteria evaluated to determine the skin status is visual perception. Consumers are concerned about the appearance of produce. Sensory evaluation of the skin status uses many words to describe appearance and the results are not objective (Jaros, Rohm, & Strobl, 2000). 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. Light reflected from the product carries information used by inspectors and consumers to judge surface characteristics related to the quality (Abbott, 1999). Veraverbeke, Verboven, Oostveldt, and Nicolai (2003a) investigated fruit surface layers with confocal laser scanning microscopy (CLSM), environmental scanning electron microscopy (ESEM), and conventional light microscopy (LM) to determine the three-dimensional structure. Similar reports include food structure analysed by CLSM (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001), fat crystals analysed by electron microscopy (Heertje & Lennis, 1997), and ice-cream analysed by optical microscopy (Caillet, Cogne, Andrieu, Laulent, & Rivoire, 2003). LM and SEM have difficulty obtaining high resolution for skin status of fine structures. Staining is needed for CLSM, and it only detects fluorescence and reflection results in a much blurred image. Gibbs and Bishop (1995) proposed using a geostatistical technique to

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describe bio-film surface roughness. Height measurement was accurate to 1 μm or less, which was a low resolution for investigating the changes of skin status. Burdon and Clark (2001) monitored the impact of water loss using serial quantitative proton magnetic resonance imaging, however, the results were not straightforward.

Recent emphasis has been placed on developing sensors for real-time sorting (Abbott, 1999). The skin status of produce was important not only for describing the sensory quality, but also for developing the kinetic model of moisture loss and gas exchange through the skin (Lammertyn, Scheerlinck, Verlinden, Schotsmans, & Nicolaï, 2001). Schotsmans et al. (2002) measured factors affecting skin resistance in pipfruit without considering the heterogeneity of skin status.

Atomic force microscopy (AFM) allows three-dimensional imaging and measurement of unstained and uncoated structures in air or fluid, permitting direct observation of native specimens and ongoing processes under native or near-native conditions.

AFM has a sharp probe attached to a cantilever spring, which is scanned in close proximity to a sample surface. During scanning, the forces between the tip and the surface cause deflections of the cantilever. A laser beam is focused onto the back of the cantilever, and by monitoring its movements, an image of the surface is built up (Allen, Davies, Roberts, Tendler, & Williams, 1997). The AFM has been used as a means for measuring roughness for plant materials (Warren & Krajcinovic, 1995; Wilbert, Pellegrine, & Zydney, 1998; Round et al., 2000), interpreting the rheology of biopolymers (Morris et al., 2001), and comparing the roughness between onion skin surface and chloroform-cleaned onion surface (Hershko & Nussinovitch, 1998a). However, there is no report about the changes of the skin status of produce during storage. Since the probe is of nanometer dimensions, the AFM might also be used to examine and discriminate between surfaces of roughness levels outside the range of other profilometers (Verran, Rowe, Cole, & Boyd, 2000).

The overall goals of this study are to propose a detecting technique for the roughness of produce skin and to examine the changes of peach skin characteristics with storage time. The reliable parameters obtained by AFM will be used to further investigate the appearance quality of produce in real time.

2. Materials and methods

2.1. Fruits

'Jinxiu' yellow peaches (*prunus persicu* L. Batsch.), at a pre-climacteric stage, were harvested in August 2003 from an orchard in Fengxian, Shanghai, China. The fruits were transported to the Laboratory of Cold Chain Research at Shanghai Jiao Tong University in 2 h and precooled (4 °C, 12 h) immediately. After cooling they were stored at 2 ± 1 °C.

2.2. Storage conditions

Storage conditions were controlled atmosphere (CA) (0.893 mmol/l O_2 + 2.232 mmol/l CO_2) and regular air (RA). One cabinet (1050 mm × 550 mm × 1000 mm) was used for CA and one for RA group. The initial concentration of O_2 and CO_2 in the CA cabinet was established by control of flow rate of N_2 generated by cellulose membrane and CO_2 via pressure regulators. Ethylene absorber worked 2 h every week and CO_2 absorber (soda lime containing ethyl violet as indicator) worked based on the CO_2 concentration displayed at an atmosphere analyzer (GAC-1100, Italy). The RA cabinet was not sealed and air in it could exchange with atmosphere. However, in order to decrease the moisture evaporation, the RA cabinet was covered to prevent peaches from circulating air directly. About 120 ± 10 kg of peaches were placed in each cabinet of both CA and RA conditions and all treatments were at 2 ± 1 °C with approximately 95% RH.

2.3. AFM measurement

The hair of the peach was carefully scoured off to eliminate the influence of the fuzz on the skin's roughness, then a thin piece of skin from the equator was removed with a knife. The sample was stuck onto a mica surface with double-sided tape, mounted onto the sample stage, then scanned at the speed of 1–2 Hz. The scanner used a multimode Nanoscope IIIa AFM (digital instruments, CA, USA) equipped with a Si_3N_4 cantilevered scanner with a $12 \times 12 \, \mu m^2$ 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. To preserve 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. This virtually eliminated lateral shearing and sample damage for the majority of specimens.

Height measurements were performed on about $2.0 \times 2.0 \, \mu m^2$ surfaces of peach skins after different storage conditions. To make the results comparable, the images were obtained from the center area of each surface not containing vasculature. Only selected representative images of each type were presented in this paper.

2.4. AFM image roughness analysis

The height variation was represented by a color scale in which bright color denoted higher areas and dark color denoted lower areas for all images. Different scales were used in the vertical and horizontal directions.

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

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

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

where $Z(i,j)$ denoted the topography data for the surface after specimen tilt-correction, Z_{ave} was the average surface height, i and j corresponded to pixels in the x and the y direction. The maximum number of pixels in the two directions were given by n_x and n_y (Lindseth & Bardal, 1999).

2.5. Statistical analysis

Since atomic force microscopes are generally limited to small scanned areas, three images of parallel samples were examined and offline analysed with AFM software (version 5.12) for each specimen in order to average the roughness value (Darrort, Troyon, Ebothé, Bissieux, & Nicollin, 1995). Roughness values were averaged and reported along with the standard deviation ($\pm$s.D.). Statistical analysis of roughness results through ANOVA ($P<0.05$) and Duncan's multiple range test were performed using SAS (Version 8.2, Cary, NC, USA).

3. Results and discussion

Fig. 1 shows the plane and three-dimensional profiles of the peach skin at initial storage. Figs. 2 and 3 show the profiles of peach skin at different storage stages (day 15, 30 and 45) of CA group and RA group.

It should be emphasized that the horizontal and vertical scales differ by around two orders of magnitude. The arithmetic roughness and root mean square roughness of these plane profiles were listed in Tables 1 and 2, respectively. In the experiments, the skin areas for studying the topology were very small and did not contain vasculature. To get good details of the profiles at vertical direction, the scale was not large enough to include all the height information, for example, in Fig. 2c and d, the maximum height was 181.70 nm (data not shown), while the vertical scale was 100 nm. Similar
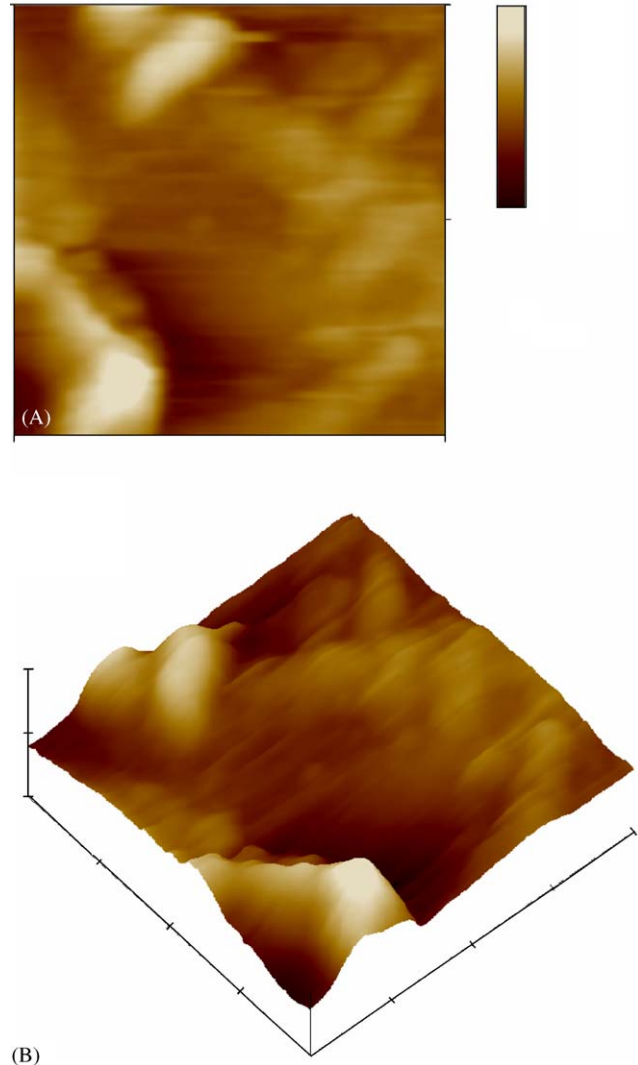

Fig. 1. Profiles of peach skin at initial storage by AFM: (A) plane profile and (B) three-dimensional profile. Scan area = 1.999 μm × 1.999 μm, height bar = 50 nm.

profiles of Figs. 2e,f to Figs. 3c,d might be the result of uncleaned tip and had effect on the next imaging.

The relationship between R_a and R_q of peach skin at different storage stages was shown in Fig. 4. The results show that R_a was in accordance with R_q with a coefficient of linear relationship 0.9979. The changes of R_a and R_q with storage time were listed in Tables 1 and 2. The results demonstrated that the different storage conditions had a strong effect on the skin roughness.

It should be stated that two groups had a similar RH and the different results in Tables 1 and 2 could indicate the velocity of moisture evaporating from the skin to the atmosphere in the cabinet. Due to different storage conditions, the different respiration rate resulted in different moisture loss, which appears as skin status.
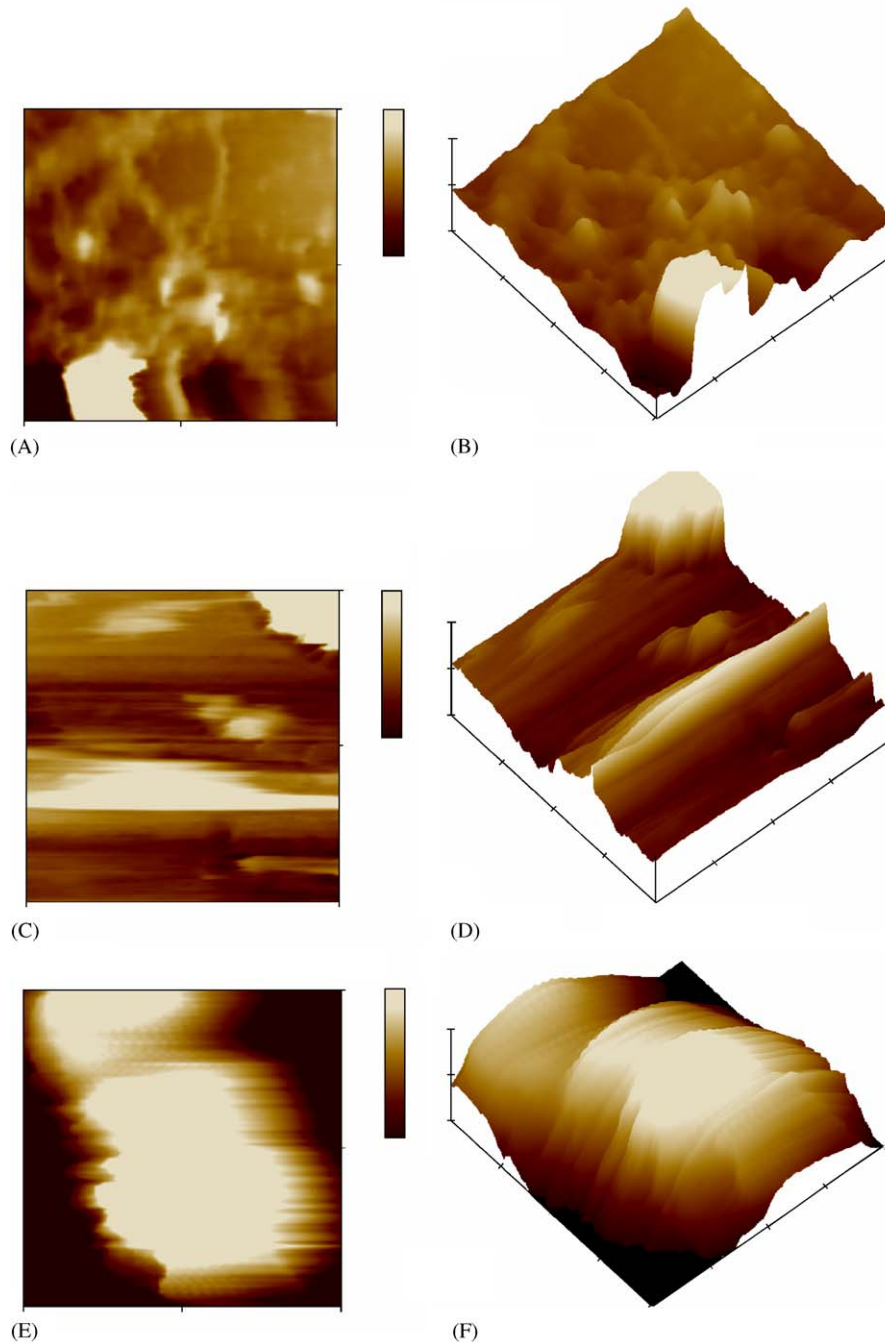
Fig. 2. Profiles of peach skin at different stage of CA storage by AFM: (A) and (B) plane and three-dimensional profiles after 15 day storage, respectively. Scan area = 2.003 μm × 2.003 μm, height bar = 50 nm. (C) and (D) plane and three-dimensional profiles after 30 day storage respectively. Scan area = 2.000 μm × 2.000 μm, height bar = 100 nm. (E), (F) plane and three-dimensional profiles after 45 day storage, respectively. Scan area = 2.004 μm × 2.004 μm, height bar = 100 nm.

The roughness analysis could be helpful to know the skin status and to further understand peach quality.

Hershko and Nussinovitch (1998a) had reported that the R_a of uncoated onion skin was 6.0 ± 3.6 μm by literature and about 78 nm measured by AFM. Similar results were reported by Hershko, Weisman, and Nussinovitch (1998b). Verran et al. (2000) reported that the roughness measured by AFM was much smaller than that measured by other methods. Roughness of certain plastics measured by surface profilometer was 0.24 ± 0.11 μm, 0.81 ± 0.06 μm by laser profilometer, and 73 ± 22 nm by AFM. This data leads us to believe that the roughness determined by AFM in our experiments would be smaller than that measured by other
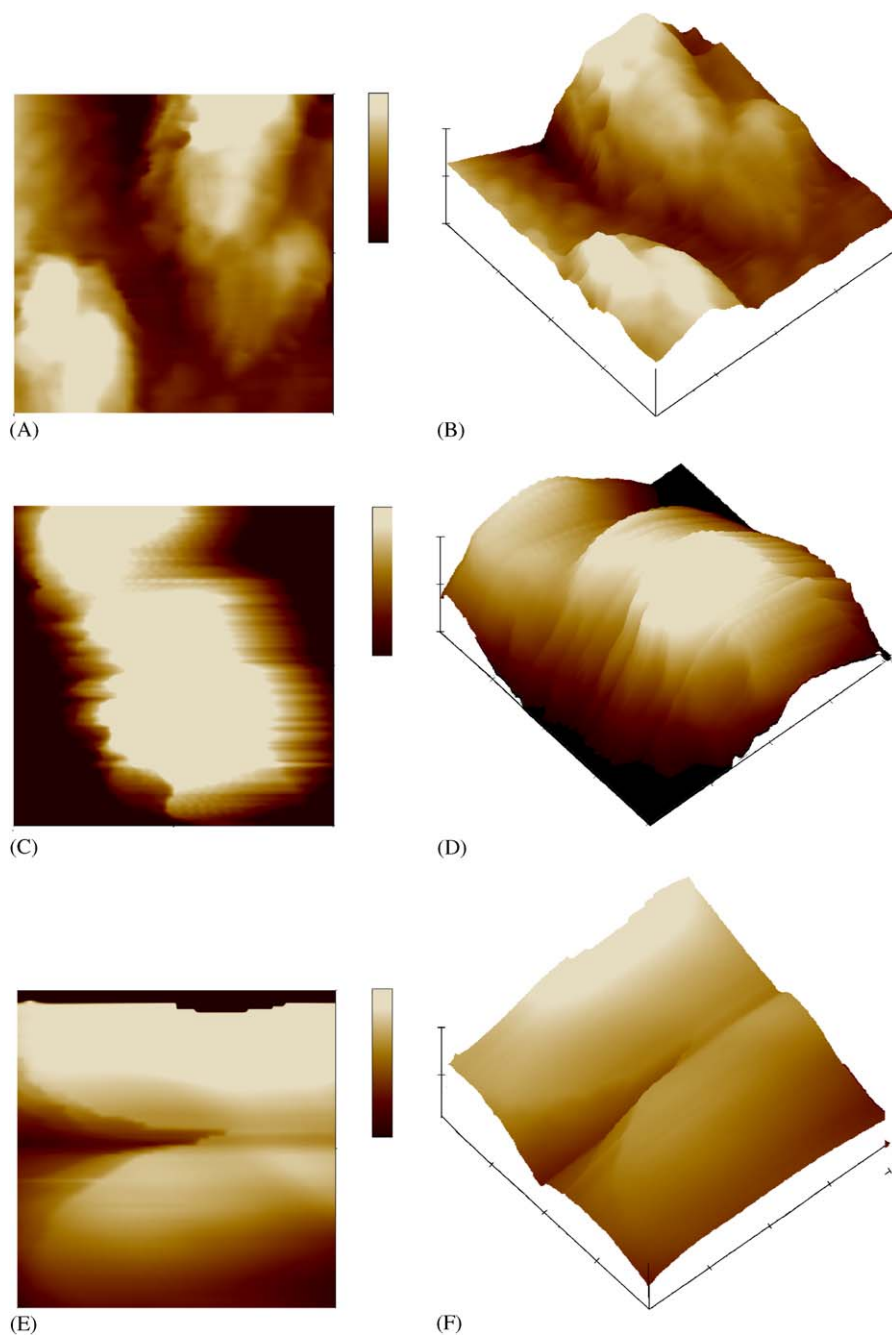
Fig. 3. Plane and three-dimensional profiles of peach skin at different stage of RA storage by AFM. (A), (B) plane and three-dimensional profiles after 15 day storage, respectively. Scan area $= 2.000\,\mu m \times 2.000\,\mu m$, height bar $= 100\,nm$. (C), (D) plane and three-dimensional profiles after 30 day storage, respectively. Scan area $= 2.003\,\mu m \times 2.003\,\mu m$, height bar $= 100\,nm$. (E), (F) plane and three-dimensional profiles after 45 day storage, respectively. Scan area $= 1.999\,\mu m \times 1999\,\mu m$, height bar $= 500\,nm$.

Table 1
Effects of storage atmosphere and time on the R_a of peach skin (nm) (mean $\pm$ SD, $n = 3$)

| Group | Days of storage (d) | | | |
	0	15	30	45
CA	6.528 ± 1.493^a	8.595 ± 3.047^a	20.829 ± 8.478^a	68.151 ± 13.376^a
RA	6.528 ± 1.493^a	19.472 ± 7.033^b	71.761 ± 17.485^b	258.333 ± 83.427^b

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

Table 2
Effects of storage atmosphere and time on the R_q of peach skin (nm) (mean$\pm$SD, $n = 3$)

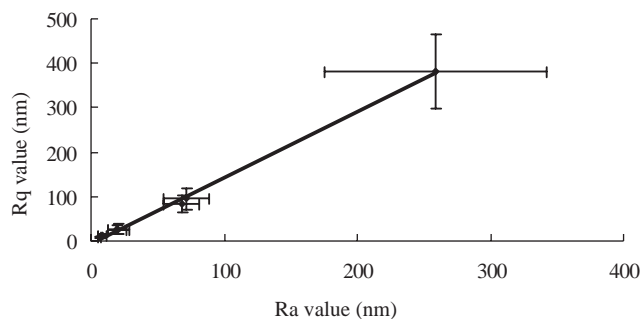| Group	Days of storage (d)			
	0	15	30	45
CA	8.084 ± 1.482^a	12.235 ± 3.487^a	26.125 ± 11.539^a	83.874 ± 18.726^a
RA	8.084 ± 1.482^a	24.893 ± 9.705^b	94.814 ± 25.257^b	380.883 ± 83.660^b

Fig. 4. The correlation between R_a and R_q of peach skin analysed by AFM. $y = 1.4818x - 5.9068$, $R^2 = 0.9979$.

Table 3
Estimation of the order of roughness changes by examining the r^2 from plots of zero, half-, first- and second-order reactions

Roughness	Group	0 order, R vs. time	1/2 order, $R^{0.5}$ vs. time	1 st order, $\ln R$ vs. time	2 nd order, $1/R$ vs. time
R_a	CA	0.7815	0.8633	0.9367	0.9813
R_a	RA	0.8033	0.9227	0.9985	0.8426
R_q	CA	0.7894	0.8762	0.9553	0.9885
R_q	RA	0.7840	0.9098	0.9977	0.8365

was decided by examining the coefficient of determination (r^2) (Lau, Tang, & Swanson, 2000). The results from graphical determination of reaction order were summarized in Table 3. The r^2 values indicated a first-order reaction of the R_a and R_q of peach skin. The reaction rate constants, calculated from the slope, of R_a for the CA and RA groups were 0.0528 and 0.0823 d^{-1}, respectively. And for R_q, the constants were 0.0518 and 0.0860 d^{-1}, respectively. The differences in rate constant of the two groups might have resulted from the inhibition of the controlled atmosphere on the evaporation of moisture, and thus the roughness value.

4. Conclusion

The roughness analysis gained from AFM was more effective in determining the roughness of peach skin during storage. AFM can be used to investigate the skin status and characteristics of peach surface. The roughness increases with the storage time, and the increasing speed of CA group is lower than that of RA group. The three-dimensional profile demonstrated that the different storage conditions had a strong effect on the skin roughness.

instruments. The value of roughness was meaningful only when the instruments were comparable.

The maximum height of unpolished onion skin reported by Hershko and Nussinovitch (1998a) was 628 nm, which was larger than that of the peach skin at initial storage with 43.221 nm (in Fig. 1a). The reason was probably that the scanned size in our tests was much smaller than that used by Hershko and Nussinovitch (1998a). It was important to understand that the roughness value depended on the scanned area and on the number of data points; roughness decreased in line with the scanned area because of the surface statistics, i.e. the fractal behavior (Darrort et al., 1995). An area of $2.0 \times 2.0\,\mu m^2$ was used to eliminate the variation from scanned size.

Regression analysis could be used to determine the reaction order of the roughness changes. The plots of R_a or R_q versus time for the zero, half, first and second orders of reaction were performed. The best fitted line

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