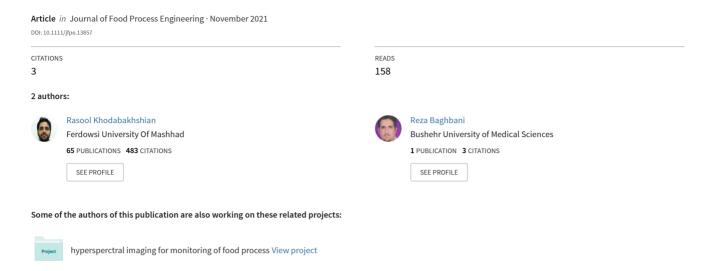
Classification of bananas during ripening using peel roughness analysis—An application of atomic force microscopy to food process



ORIGINAL ARTICLE

Classification of bananas during ripening using peel roughness analysis—An application of atomic force microscopy to food process

Rasool Khodabakhshian¹ Reza Baghbani²

¹Department of Biosystems Engineering, Ferdowsi University of Mashhad, Mashhad, Iran

²Department of Agricultural Engineering, Technical and Vocational University (TVU), Tehran, Iran

Correspondence

Rasool Khodabakhshian, Department of Biosystems Engineering, Ferdowsi University of Mashhad, Mashhad, Iran. Email: khodabakhshian@um.ac.ir

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Abstract

To monitor banana surface (peel) roughness changes during ripening treatment, atomic force microscopy (AFM) as a novel and emerging technique was used in this study. The roughness of banana peel was studied using the arithmetic mean between peaks and troughs (R_a) and the root-mean-square roughness (R_a) . It was concluded that with changing the ripening stages, the behavior of the roughness changes significantly. With advancing fruit ripening, the extending of the epicarp and the decrease of the surface (peel) roughness were found due to enlarging of fruit volume. The highest mean roughness was found to be at stage 1, $R_a = 8.25$ and $R_q = 9.65$ nm. Based on two-dimensional profile results, the surface (peel) roughness was affected strongly by studied different ripening stages. It was concluded that the peak values in the ripple profiles become smaller with advancing fruit ripening. However, the effects of noise in the profiles appeared to increase. So, in all the initial stages of banana fruit ripening, the noise was found to be minimal. Consequently, the AFM technique was found to be a promising tool for quantification of the peel roughness or glossiness and also could help in the quality control of banana fruit on the nanoscale.

INTRODUCTION 1

Banana (Musa sap.) fruit is well-known due to its high nutritional value and as a climacteric fruit that continues to ripen after harvest (Salunkhe, 1995). Hence, an assessment of its quality during the ripening of fruits is very necessary. General criteria for judging ripeness consist of physiological parameters, such as peel/skin attributes, texture, geometrical (such as size and shape), gravimetrical (such as moisture contents and weight), and chemical characteristics (such as total soluble solids, pH, dry weight content, and acidity) (Gomes, Vieira, & Leta, 2013; Mim, Galib, Hasan, & Jerin, 2018). Among these, peel/skin attributes have been distinguished as a suitable ripeness parameter for numerous vegetables and fruit, such as pomegranate, tomato, cucumber, strawberry, banana, and so forth. Moreover, the peel/skin attributes a suitable factor in the design of packing processes and sorting machines, and also, it usually provides an effective parameter for crop growing management. While fruit is on the tree, its peel/skin

is suitable to evaluate ripeness and provide harvest time forecasting (Mim et al., 2018). Similarly, this parameter (peel/skin attributes) is used by food and agricultural industries to assess the ripeness index.

As a gradual process pertinent to chlorophyll pigment degradation, the banana peel color changes from the initial (with green color) to ending ripeness stage (with yellow color), with brown spots emerging on the yellow color at the last stage of ripening (Gomes et al., 2013; Mim et al., 2018). In the banana industry, it is very necessary to classify the bananas before the packing for the market as those ripeness stages specify the shelf life (CEAGESP, 2006). Among the factors assessed in banana classification (producer identification, group, class, subclass, etc.), the one that presents the maximum complexity is specifying the subclass, which assesses the bananas during ripening. In the trade market, seven ripening stages of banana fruit based on the Von Loesecke ripening scale are usually discerned.

Von Loesecke (1949) specified seven ripeness subclasses, C1-C7, relating individually to the following peel color phases: C1 = thoroughly

green peel; C2 = green peel with traces of yellow; C2 = more green than yellow; C3 = more yellow than green; C4 = yellow peel with traces of green; C5 = thoroughly yellow peel; and C7 = yellow with brown spots (Gomes et al., 2013; Mim et al., 2018; Soltani, Alimardani, & Omid, 2011). Traditionally, the assessment of the subclass is performed through visual inspection, based on a series of color charts of the different ripeness stages by specialists (Gomes & Leta, 2012). This comparative method is vulnerable to mistakes due to the distraction, tiredness, and tedium of the specialists. Also, it is extremely hard for human experts to classify a large number of bananas physically and pack them likewise. Therefore, it is essential to develop an automated system of bananas ripeness classification, so that the banana industry workers could be able to handle many bananas in a very short time and with diminished expense in an easy effective application.

Most fruit ripeness classification studies and postharvest fruit quality that have been investigated by researchers are fruit firmness, machine vision, and spectroscopy at the macro-scale (Sun, 2016; Hussain, Pu, & Sun, 2017; Khodabakhshian et al., 2017; Khodabakhshian and Emadi, 2017; Mim et al., 2018; Czieczor, Bentkamp, Damerow, & Blanke, 2018; Pathmanaban, Gnanavel, & Anandan, 2019; Khodabakhshian et al., 2019; Khodabakhshian, 2019; Sinambela, Mandang, Dewa MadeSubrata, & Hermawan, 2020). However, research at the micro- and nano-scales has been constrained by the absence of innovation. Some research reports are transmission/scanning electron microscopy (TEM/SEM) on fruit and vegetables (He, Feng, Yang, Wu, & Li, 2004), light microscopy (LM) on ice cream (Caillet et al., 2003), confocal laser scanning microscopy (CLSM) on fruit surface layers (Veraverbeke, Verboven, Oostveldt, & Nicolai, 2003), and cellular force microscope (CFM) on fruit and vegetables (Routier-Kierzkowska et al., 2012). However, this strategy experiences issues for skin status of fine structures. When these techniques are used to measure luster, color changes and surface observations require a flat surface. On fruit like banana, curved color variation from the center to the tips needs to be measured in many positions to create a representative profile. This shortcoming can now be overcome by atomic force microscopy (AFM). AFM has recently been recognized as a powerful tool that allows three-dimensional imaging and also as equipment for measuring roughness for biomaterials (Cybulska, Zdunek, Psonka-Antonczyk, & Stokke, 2013; Akhatova, Fakhrullina, Khakimova, & Fakhrullin, 2018; Panchal, Fakhrullina, Fakhrullin, & Lvov, 2018; Cárdenas-Pérez et al., 2019; Posé et al., 2019; Cavallaro et al., 2020; Khodabakhshian et al., 2021; Khodabakhshian and Hassani, 2021).

However, no report has been published for the surface changes of agricultural products during ripening or postharvest by AFM. So, the main goal of this research is to offer a quantitative procedure for the roughness of agricultural products and to inspect the variations of banana surface (peel) attributes when assessing the ripeness stage of bananas during ripeness treatment, and also proposes to apply this method to an automated control system for the ripeness process of bananas.

2 | MATERIALS AND METHODS

2.1 | Fruit source

The banana fruit (cavendish variety) at different ripening stages (seven ripening stages) was used for this study. The bananas were visually checked for appearance and surface defects, and finally, 100 fruits free from any abnormal features were randomly picked from banana bags in a ripeness chamber of Meraj warehouse situated in Mashhad city in Khorasan Razavi, Iran. The test was done at airtight ground warehouses located in Bu Ali Research Institute, Mashhad, Iran, with bananas preserved at the moisture content of 85-88% for 5 days, the time required for processing the ripeness treatment of bananas. Based on the Von Loesecke standard, visually six ripeness stages were evaluated by comparing the peel color of samples (Figure 1). Right from the start (first day), bananas were at Stage 1, and Stage 6 was accomplished on the fifth day. On the first day, ethylene gas with a concentration of 1.000 mg/L was applied for about 24 hr. Stage 7 is not completed in the warehouse, and at the end of Stage 6, the bananas are transported to the market. So, fruit samples at Stage 7 were selected from local supermarkets without blemishes.

2.2 | Roughness parameters measurement (AFM studies)

2.2.1 | AFM measurement

Five thin pieces of peel were randomly extracted from the 10 regions (with a distance of 15 mm from each other) of the banana surface at each seven studied ripening stages; the five measurements was done on these spots (Figure 2). So, for each banana at seven studied ripening stages, 250 measurements were performed. The five sample banana fruits studied correspond to seven studied ripening stages, and the average of these five measurements was used to represent ripple profile of banana surface at seven studied ripening stages. The samples were studied for surface roughness with an AFM contact mode (NanoWizard II, JPK Instruments, Berlin, Germany) with $100 \times 100 \text{ nm}^2$ scan range and 10k pixels and a nominal tip (FluidFM tip) radius of 10 nm and a 0.7 N/m nominal spring constant (JPK Instruments).

All tests were carried out perpendicular to the longitudinal axis of the fruit sample. The scanning was carried out at a 1–2 Hz speed. But, before this procedure, the samples were fixed onto a surface (mica) with double-sided tape, mounted onto the sample stage. Before capturing the tip with the banana surface, a 0.3 V set point and a scan size were set to 0 nm. Afterward, the capturing of the tip with the banana surface was done automatically. Once the tip was captured with the sample, the set point was set back to -10 V, which led to retraction of the tip from the banana surface. Thereafter, in this situation, an establishment of a scan size of 10 $\mu m \times 10~\mu m$ (a size of 512×512 pixels) and scanning of a blank image was carried out. Finally, a 1 V set point was fixed, which led to the extension of the

FIGURE 1 Von Loesecke ripening scale (CEAGESP, 2006): Stage 1 = thoroughly green peel; Stage 2 = green peel with traces of yellow; Stage 3 = more green than yellow; Stage 4 = more yellow than green; Stage 5 = yellow peel with traces of green; Stage 6 = thoroughly yellow peel; Stage 7 = yellow with brown spots

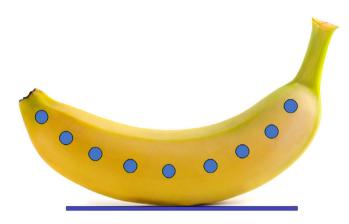


FIGURE 2 A position of banana during roughness measurements

AFM tip to the sample, and then, a regular matrix including 16 points on the scan was signed (4 \times 4 points). Ten indentations were carried out on each point. Roughness assessment was carried out in the JPK SPM software (Bruker, NT-MDT, etc.). The whole process from setting till getting the results of data analysis was 5 min for each sample.

2.2.2 | AFM image roughness analysis

The procedure used for the roughness analysis was based on a published method (Yang, An, Feng, & Li, 2005) with small modifications. In brief, a color scale was used to present height variation in which higher areas were specified by bright color and lower areas designated by dark color for all images. Both vertical and horizontal directions were depicted with various scales. Two parameters (amplitude and average) were studied. The arithmetic mean between peaks and troughs, $R_{\rm a}$, and the root-mean-square (RMS) roughness, $R_{\rm q}$, were assumed by:

$$R_{a} = \frac{1}{n_{x}n_{y}} \sum_{i=1}^{n_{x}} \sum_{i=1}^{n_{y}} |Z(i,j) - Z_{ave}|, \tag{1}$$

$$R_{q} = \sqrt{\frac{\Sigma_{i=1}^{n_{x}}\Sigma_{j=1}^{n_{y}}[Z(i,j) - Z_{ave}]^{2}}{n_{x}n_{y}}}, \tag{2}$$

where the topography data of the surface after specimen tilt correction were specified with Z(i,j), the average surface height was

designated with Z_{ave} , i and j corresponded to pixels in the x- and y-directions, and n_x and n_y specified the maximum number of pixels in the x- and y-directions. Also, as many researchers have reported, besides the vertical roughness, the lateral roughness fluctuations are important. So, the lateral correlation length of roughness was calculated based on the method by Czibula et al. (2019).

2.2.3 | Statistics

As was stated in Section 2.2.1, 160 roughness information was gathered for each cell in the method stated in this section, that is, the matrix of 16 points (10 information for each point). Roughness properties were determined individually for each point, and then, the mean (±SD) values were reported. Roughness analysis was carried out in the JPK SPM software (Bruker, NT-MDT, etc.). The mean values of roughness were stated by the standard deviation (±SD). The analysis of variance (ANOVA) using SPSS 16.0 software was used for statistical analysis of roughness results. To separate means at a 5% level of significance, Duncan's multiple ranges tests were used.

3 | RESULTS AND DISCUSSION

3.1 Visual quality assessment of fruit

As it can be seen from Figure 1, an increase in the glossiness with advancing fruit ripening is perceived, being a glossy and smooth peel at the full-ripe stage in a ripening chamber (Stage 6). As it is stated in the next section, a smooth and shiny appearance displays a low value of peel roughness. In agreement with these results, Ringer, Damerow, and Blanke (2018) using the luster sensor reported that the glossiness increased from Stage 3 (green) to Stage F7a (ripe) of ca. 35%, followed by a decrease in glossiness from Stage F7a to Stage F7b (overripe).

3.2 | Roughness analysis

As it was stated earlier, in this study, the roughness was quantified by two parameters to determine banana surface (peel) roughness, the arithmetic means between peaks and troughs (R_a), and the RMS roughness (R_a). Based on EN ISO 25178, both R_a and R_q are

exclusively appropriate for the descriptive statistics and comparative analysis of surfaces and have been studied by other researchers for fruit (Czieczor et al., 2018; Klemm, Damerow, & Blanke, 2016; Ringer et al., 2018; Wang, Feng, Liang, Luo, & Malyarchuk, 2009; Yang et al., 2005).

Figure 3 shows the typical two-dimensional images and ripple profile of the banana surface at seven studied ripening stages. Each of these profiles was generated by selecting one line from the corresponding two-dimensional AFM scan and subsequently plotting x translation versus height data. Each profile was plotted using an appropriate height scale to provide optimum resolution. There was a relatively low signal-to-noise ratio in the surface

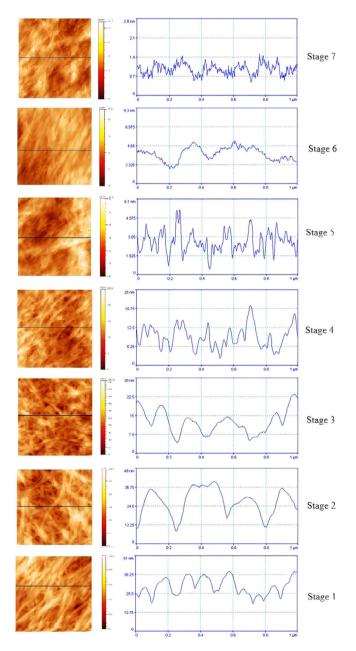


FIGURE 3 Typical two-dimensional images and ripple profile of banana surface at seven studied ripening stages, based on $1~\mu m \times 1~\mu m$ atomic force microscopy scans

profile of the fruit at Stage 7, and to a lesser extent in that of the fruit at Stage 6, but in all of the surface profiles, the contribution of the noise was found to be minimal. As it can be found from this Figure, peaks in the profiles become smaller with advancing fruit ripening.

Similar to Ringer et al. (2018), the results showed that differences at a 5% level of significance were found among seven studied ripening stages in the roughness measurements (results not shown). The Ra and R_q of these ripple profiles appear in Table 1. As can be seen from this Table, the samples with the highest mean roughness were found to be at Stage 1, $R_a = 8.25$ and $R_g = 9.65$ nm. They both measured the values of the R_a and R_q , which decreased significantly with advancing banana fruit ripening and, thus, confirmed the visual quality assessment in Section 3.1. The 2.6-fold increase in R_a was reported by Ringer et al. (2018) of 2.5-6.5 µm during ripening Stage 3 to Stage 7b. To date, no report has been published for the R_{α} measurement during banana fruit ripening, so the discussion does not apply. According to Hershko and Nussinovitch (1998), the R_a of uncoated onion skin was reported $6.0 \pm 3.6 \mu m$ by literature and about 78 nm by AFM. Yang et al. (2005), Cardona, Oliveros, Arias, Alvarez, and Devia (2008), Czieczor et al. (2018), and Ringer et al. (2018) reported that the roughness measured by AFM was lower than that measured by other methods for agricultural products. Based on literature review by the authors, the surface profilometer, laser profilometer, and AFM showed the roughness of certain plastics at $0.24 \pm 0.11 \,\mu m$, 0.81 \pm 0.06 μ m, and 0.73 \pm 0.22 nm, respectively. These results conduct us to believe that the roughness determined by other apparatuses would be larger than measured by AFM in our research. The estimation of roughness was meaningful just when the instruments were practically

On the other hand, the maximum height of peach skin reported by Yang et al. (2005) was 43.221 nm, which was larger than that of the banana surface in our study. The reason for these different values may be attributed to the scanned size in our experiments, which was much smaller than that used by Yang et al. (2005). It was critical to get it that the roughness value depended on the scanned area and the number of data points (Darrort, Troyon, Ebothe, Bissieux, & Nicollin, 1995). To eliminate the variation from scanned size, an area of $5\times 5~\mu m^2$ was utilized.

Based on DIN EN ISO 4287, both R_a and R_q that have a place with Category 2 (amplitude parameters and average) are specified by estimating track. R_q is less utilized as a measurement parameter to study surface roughness, but more meaningful than R_a . Significantly, there are additional parameters to study roughness, which could be valuable in numerous fields of application, for instance, R_z . According to DIN EN ISO 4287, this parameter (R_z) fits Category 1 (amplitude parameters, peak heights, and depths). The average parameters $(R_a$ and $R_q)$ are less susceptible to extreme profile peaks and troughs due to individual peaks and troughs that have a small impact on the obtained value. On the other hand, R_z is the result of the sum of the largest profile peaks and profile depths and better reflects the roughness attributes as for irregular deviations. Anyway, one thing is basic to all—they are not appropriate for the absolute evaluation and the

TABLE 1 Influence of studied ripeness stages on the R_a and R_q of banana surface (peel; nm; mean \pm SD); the data are obtained from analysis of the 1 μ m \times 1 μ m topography images

	Ripening stages						
Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7
R _a	8.25 ± 0.02	6.08 ± 0.03	5.23 ± 0.04	3.13 ± 0.04	2.32 ± 0.03	1.43 ± 0.05	1.12 ± 0.04
R_{q}	9.65 ± 0.03	7.50 ± 0.03	7.05 ± 0.02	4.56 ± 0.03	2.98 ± 0.05	1.78 ± 0.04	1.12 ± 0.04

TABLE 2 Influence of studied ripeness stages on the lateral correlation length of the roughness of the banana surface (peel; nm; mean \pm SD); the data are obtained from analysis of the 1 μ m \times 1 μ m topography images

	Ripening stag	Ripening stages							
Parameter	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7		
Lateral correlation length [nm]	335 ± 20	280 ± 15	225 ± 10	185 ± 15	155 ± 10	110 ± 10	85 ± 5		

TABLE 3 Assessment of the order of roughness changes by investigating the correlation coefficient (r^2) from plots of zero-, half-, first-, and second-order reactions

	Order values						
Parameter	Zero order, R versus ripening	Half order, R ^{0.5} versus ripening	First order, InR versus ripening	Second order, 1/R versus ripening			
R_a	0.73	0.90	0.99	0.96			
$R_{\rm q}$	0.73	0.89	0.99	0.95			

comparative evaluation. The more parameters are utilized, the more accurate the surface estimations.

Also, as it can be seen from Table 2, the samples with the highest and lateral correlation length were found to be at Stage 1 (335 nm) and Stage 7 (85 nm), respectively. Similar to roughness results, this parameter decreased significantly with advancing banana fruit ripening and, thus, confirmed the visual quality assessment in Section 3.1, too. A similar trend was reported by Czibula et al. (2019) at design of friction, morphology, wetting, and protein affinity by cellulose blend thin-film composition.

To the reaction order of the roughness change, regression analysis might be applied. The plots of $R_{\rm a}$ or $R_{\rm q}$ as opposed to ripening stages for the zero, half, first, and second orders of reaction were carried out. The best model was selected by investigating the correlation coefficient (Clark et al., 2004). Table 3 presents the results from the graphical designation of reaction order. The best-fitted line for the determination of roughness change was achieved when the first-order reaction of the banana surface $R_{\rm a}$ and $R_{\rm q}$ was applied. The obtained correlation coefficients for best model were $r^2=0.99$ and $r^2=0.99$ for $R_{\rm a}$ and $R_{\rm q}$, respectively.

4 | CONCLUSION

In this study, the feasibility of using AFM as a novel and emerging technology to examine surface (peel) roughness in banana fruit when assessing the ripeness stage of bananas during ripening treatment was studied.

These findings are valid to other kinds of biomaterials in which a glossy, smooth peel is also a quality attribute. The following conclusions were obtained from this study:

- 1. It was demonstrated that the AFM technique is a promising tool for quantification of the peel roughness or glossiness and also could help in the quality control of banana fruit on the nanoscale.
- 2. There is concluded that with changing the ripening stages, the behavior of the roughness changes significantly.
- 3. The glossiness increased with advancing fruit ripening, being a glossy and smooth peel at the full-ripe stage in a ripening chamber.
- 4. The roughness was quantified by two parameters to determine banana surface (peel) roughness, the arithmetic mean between peaks and troughs (R_a), and the RMS roughness (R_a).
- 5. The two-dimensional profile depicted that the different ripening stages had a strong effect on the surface (peel) roughness.
- 6. The peak values in the ripple profiles become smaller with advancing fruit ripening. However, the effects of noise in the profiles appeared to increase. So, in all of the initial stages of banana fruit ripening, the noise was found to be minimal.
- 7. The measured values of the arithmetic roughness (R_a) and RMS roughness (R_q) both decreased significantly with advancing banana fruit ripening, and the highest mean roughness was found to be at Stage 1, $R_a = 8.25$ and $R_q = 9.65$ nm.
- 8. To the reaction order of the roughness change, regression analysis might be applied. The best-fitted line for the determination of roughness change was achieved when the first-order reaction of the banana surface R_a and R_q was applied.

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CONFLICT OF INTEREST

The authors declare that he has no conflict of interest.

AUTHOR CONTRIBUTIONS

Rasool Khodabakhshian: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **Reza Baghbani:** Writing – review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

ORCID

Rasool Khodabakhshian https://orcid.org/0000-0003-3549-9668

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