Effect of Surface Roughness on Retention and Removal of *Escherichia coli* O157:H7 on Surfaces of Selected Fruits

H. WANG, H. FENG, W. LIANG, Y. LUO, AND V. MALYARCHUK

ABSTRACT: This study was undertaken to evaluate the effect of surface roughness on the attachment and removal of *Escherichia coli* O157:H7 on selected fruit and metal surfaces. A new method to determine surface roughness was developed using confocal laser scanning microscopy (CLSM). A series of 2-D layered images were taken by CLSM optical slicing of the surfaces of Golden Delicious apples, navel oranges, avocadoes, and cantaloupes. The average roughness (R_a) of the fruit surfaces was assessed by reconstructing a series of 2-D images into 3-D images. A cocktail of 5 *E. coli* O157:H7 strains were spot inoculated onto fruit skin surfaces with different R_a. The fruits were then treated with acidic electrolyzed water (AEW), peroxyacetic acid (POAA), and sterilized deionized water. Aluminum stubs with different R_a values as a model system were also spot inoculated with *E. coli* O157:H7 and subjected to a sonication treatment. Test results indicated that there was a positive linear correlation between R_a and adhesion rate of *E. coli* O157:H7, and a negative correlation between R_a and the efficacy of inactivation by AEW and POAA, respectively, on fruit surfaces. A linear increase of residual bacteria population with increased surface roughness of aluminum stubs was also observed. The relationship between surface roughness and surface hydrophobicity was negative linear for the aluminum stubs, but was quadratic for the 4 fruits. The environmental scanning electron microscopy images showed that bacteria tended to attach to or be entrapped in the grooves or cavities of fruits, which provided protection to the cells against washing treatments.

Keywords: confocal laser scanning microscopy, electrolyzed water, hydrophobicity, produce washing, surface roughness

Introduction

Since its discovery in 1982, Enterohemorrhagic *Escherichia coli* O157:H7 has emerged as a highly significant foodborne pathogen due to the role it played in a number of foodborne illness outbreaks (Hilborn and others 1999). To address the microbial safety problem associated with this organism, studies have been conducted to examine the efficacy of chlorine, hydrogen peroxide, ozone, peroxyacetic acid (POAA), and acidic electrolyzed water (AEW), among others, on inactivation of *E. coli* O157:H7 on fresh produce (Park and Beuchat 1999; Wright and others 2000; Park and others 2001; Yu and others 2001; Sharma and others 2002; Koseki and others 2004; Wang and others 2006, 2007). A comparison of these studies reveals that the same sanitizer at the same concentration can have different efficacy on bacterial population reduction when applied to different produce items. Since most produce washing operations are surface treatments, the differences in the microbial count reduction may be attributed to the differences in surface conditions and surface characteristics among the produce.

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The surface properties of plants can be described by surface hydrophobicity, surface constitutional characteristics, and surface topography. The surface hydrophobicity relates to surface chemical composition and surface structures, and influences surface hydration (Vacheethasanee and others 1998). The correlations between surface hydrophobicity and surface roughness for stainless steel and titanium oxide have been studied by Boulangé-Petermann and others (1997) and Lee and others (2000), respectively. However, there is no report about the effect of surface hydrophobicity of fruits and vegetables on bacterial adhesion. The relationship between surface hydrophobicity and surface roughness for fresh produce is largely unknown.

Among the surface constitutional characteristics, the cuticles are usually embedded with cuticular wax which not only influences plant surface hydration, but it also alters the interaction between a plant and microorganisms (Beattie and Marcell 2002). The stomatas, lenticels, broken trichomes and cuticle, and scars on the plants provide natural pathways for the entry of microorganisms (Mansvelt and Hattingh 1987; Babic and others 1996; Seo and Frank 1999).

The surface topography of fruits and vegetables can be quite complex, because there are multiple scales of topography that contribute to the overall topography. The topography at a stereomicroscopic scale is dictated by huge undulations on the surface that involve tens or hundreds of cells, almost like huge cavernous valleys. At a more local scale, topography is represented by the shape and curvature of the anticlinal walls of individual epidermal cells. Both scales may impact the ability of a water drop and hence bacteria (assuming in aqueous state) to arrive on any particular

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region of the surface. At an even smaller scale like that observed with a scanning electron microscope (SEM), topography is determined by roughness on the surface of individual epidermal cells. At this scale, the surface roughness and surface hydrophobicity strongly influence the movement of the water/bacterial suspension, and thus the distribution of bacteria.

The surface topology has been found to influence the bacterial attachment to and removal from a surface. Surface irregularities of polymeric materials or stainless steel have been reported to promote bacterial adhesion and biofilm deposition whereas ultrasmooth surfaces seemed to reduce the possibility for microbial attachment (Boulangé-Petermann and others 1997; Scheuerman and others 1998; Faille and others 2000; Jullien and others 2002). Han and others (2000) observed that *E. coli* O157:H7 more preferentially attached to coarse, porous, or injured surfaces than uninjured surfaces of green peppers. Liao and Sapers (2000) suggested that the greater attachment of *Salmonella* Chester to injured tissue of apples than to unbroken skin is due to the differences in topographical structures and specific physicochemical properties.

Topography can be quantified by roughness parameters such as average roughness (R_a) (Verran and Boyd 2001; Verran and others 2001). There is a variety of roughness measuring instruments, including surface profilometers and atomic force microscopy (AFM), 2 contact mode measurement methods. With these contact methods, however, it is difficult to measure the roughness of soft, deformable, and rough surfaces of fresh fruits and vegetables. Several methods, including the glistening point method (Quevedo and Aguilera 2004) and the fractal image texture analysis (Pedreschi and others 2000; Quevedo and others 2002), have been studied to quantitatively describe surface roughness of foods. Confocal laser scanning microscopy (CLSM) is a relatively new optical tool for noninvasive evaluation of microstructures of foods and other materials. The primary value of the CLSM lies in its ability to make a series of observations (2-D layered images) within a 3-D specimen by focusing at different heights of the specimen, in a process called optical slicing (Rao and others 1992). The specimen can be illuminated and imaged through a pinhole to remove out-of-focus light, thus producing a clearer image (Kaláb and others 1995). CLSM has been used to obtain 3-D information on the cellular structure of yam parenchyma and the properties of protein and starch networks of wheat products (Dürrenberger and others 2001).

The objectives of this study were to develop a noncontact method with CLSM to quantitatively measure the surface roughness of fresh produce, and to examine the effect of surface roughness on the attachment and removal of *E. coli* O157:H7 on surfaces of selected fruits.

Materials and Methods

Surface roughness determination

Sample preparation. Golden Delicious apples, navel oranges, avocadoes, and cantaloupes were purchased from a local supermarket and stored at 4 °C. The fruits were brought to room temperature before sample preparation. The fruit surfaces with skin attached were cut into disks (40 mm in diameter) and then enclosed with skin up in a transparent Petri disk with a piece of wet paper on the bottom. The disk was then sealed with a tape to prevent water loss during measurement.

2-D image acquirement with CLSM. Confocal laser scanning microscopy (WITec alpha, Ulm, Germany) available in the Laser and Spectroscopy Facility at the Material Research Laboratory of Univ. of Illinois at Urbana-Champaign was used to take a series of 2-D images (100 × 100 μm) by optically slicing the sample surface. The separation between observation planes was set at 0.05 μm for apple and 2 μm for orange, avocado, and cantaloupe. The observation depth was 6.4 μm for apple and 200 μm for orange, avocado, and cantaloupe. The image layers were scanned from top to bottom and left to right. The magnification was approximately 2000 times. In total, 128 and 101 adjacent planes (images) were acquired for apple and other fruits, respectively.

3-D image reconstruction. Each 2-D image created by the optical slicing method was composed of light points with different light intensities. For any point on a sample surface with coordinates of (x, y), one can identify a corresponding point with the same coordinates on each slicing plane. However, for each coordinate, there is only 1 plane on which the light intensity has maximal value. Maximal light intensity is achieved on each slicing plane only at the very surface of the sample, where the light beam emitted from CLSM is reflected maximally. If the height of the coordinates corresponding to maximal light intensity can be recorded, one can produce a surface profile of the sample from which the roughness information can be extracted.

Let $T_i(x, y)$ stand for the light intensity at point (x, y) on a 2-D image. The slicing plane on which the maximal light intensity at (x, y) is achieved can be found with the relation:

$$I(x, y) = \arg_i \max T_i(x, y) \tag{1}$$

where $i = 1 - 128$ for apples and $i = 1 - 101$ for other fruits, and the function arg max () searches for argument i that corresponds to the plane which has the maximum light intensity. $I(x, y)$ is a 2-dimensional array storing the plane number corresponding to the maximal light intensity for point (x, y). The height of a point (x, y) on the sample can thus be determined by the following equation:

$$h(x, y) = I(x, y) \cdot \Delta h \tag{2}$$

where Δh is the height between 2 adjacent slicing planes along the z-axis.

Roughness calculation. The surface profile information for fruits was obtained from the 3-D reconstruction and expressed by parameter R_a, the arithmetic average of the absolute values of the surface height deviations measured from the mean plane. R_a is given by:

$$R_a = \frac{1}{N} \sum_x \sum_y Z(x, y) \tag{3}$$

$$Z(x, y) = |h(x, y) - M| \tag{4}$$

$$M = \frac{1}{N} \sum_x \sum_y h(x, y) \tag{5}$$

where $Z(x, y)$ was the distance from each point to the mean plane, M was the height of the mean plane, and N was the total number of points on each image. The determination of $h(x, y)$ and R_a was done by a program composed using Metlab.

Verification of the surface roughness determination method. A piece of metal (Nickel) with a nominal surface roughness of $R_a = 1.60 \pm 0.16$ μm was provided by Rubert Co. LTD (Cheadle, U.K.) and chosen as a specimen in the test. The 3-D topography of the metal surface was acquired with the newly developed CLSM method described previously and the R_a value

of the specimen was calculated using Eq 3 to 5. During the CLSM measurement, the separation distance between 2 planes was set at 0.05 μm, and the observation depth was 6.4 μm. In total, 128 adjacent planes (2-D images) were acquired and used to reconstruct the 3-D image of the metal surface. The R_a value of the specimen was also measured with a Sloan Dektak 3 ST stylus surface profilometer (Sloan Tech. Corp., Goleta, Calif., U.S.A.). The scan length, scan speed, and stylus force of surface profilometer were set at 100 μm, low, and 15 mg, respectively. The R_a value was estimated automatically by a computer connected to the surface profilometer. The measurement was repeated 6 times.

Influence of surface topography on *E. coli* O157:H7 removal

Inocula preparation. A 5-strain cocktail of *E. coli* O157:H7 (13B88, apple juice isolate; G5303, apple cider isolate; C7927, apple cider isolate; 204P, pork isolate; EDL933, Human feces isolate) was used in the study. Cultures were transferred 3 times to tryptic soy broth (pH 7.3, Difco Lab, Detroit, Mich., U.S.A.) by loop inoculation at successive 24-h intervals and incubated at 37 °C. Twenty-four-hour bacterial cells were harvested by centrifugation (10000 × *g*) at 4 °C for 10 min. The cell pellets were washed twice in salt peptone (0.85% NaCl, 0.1% Bacto Peptone), and resuspended in 10 mL of 0.1% peptone water. Equal volumes (2 mL) of 5 cultures were mixed to obtain an inoculum containing approximately 10^{10} CFU/mL *E. coli* O157:H7.

Sample preparation. The polished outer surfaces of aluminum stubs (SPI supplies, Div. of Structure Probe Inc., West Chester, Pa., U.S.A.) were scraped with different gauges of sandpaper to create a series of surface roughness values corresponding to different types of fruit surfaces. The surface roughness of these aluminum stubs was measured with the surface profilometer. Golden Delicious apples, navel oranges, avocadoes, and cantaloupes were sanitized with UV light for 20 min to inactivate naturally occurring microorganisms on the surfaces. A sterilized brass cork borer (Nr 9, 15.5 mm D) was used to prepare fruit plugs with skin, and a sterile knife was then used to cut fruit plugs into disks (3 to 4 mm in thickness). The disks were prepared from different areas of the same fruits. After preparation, disks were pooled together, and 5 disks from the pool were randomly selected and used as a composite sample. Each composite sample of one kind of fruit was used for one experiment. Experiment was replicated 3 times. The aluminum stubs and fruit disks were spot inoculated with 20 μL (8.30 $\log_{10}$ CFU) of the 5-strain cocktail of *E. coli* O157:H7. To facilitate drying, the 20 μL were deposited in 10 small drops on the aluminum stubs and fruit disk surfaces. All inoculated samples were air dried for 2 h in a laminar flow biological hood before treatments.

Treatments. The surface topography of stainless steel is an important physical factor influencing the hygienic condition of stainless steel in the food industry (Boulangé-Petermann and others 1997; Faille and others 2000; Jullien and others 2002). However, the narrow range in surface roughness of commercially available stainless steel makes it difficult to obtain a correlation between bacteria population adhesion and the degree of roughness. For instance, the study on *Pseudomonas aeruginosa* adhesion to stainless steel showed no clear relationship between the number of adhering cells and average roughness in the range of 0.015 to 1.04 μm (Boulangé-Petermann and others 1997). To examine the effect of R_a on microbial removal on metal surfaces, aluminum stubs with a relatively wide range of manually created surface roughness values were used in this study.

Each of the inoculated aluminum stubs was sonicated in 20 mL sterilized deionized water in a sterilized 50 mL beaker for 2 min at 40 kHz (Crest Ultrasonics, Conn., U.S.A.). Inoculated fruit disks were washed in a beaker at room temperature for 5 min with 50 mL of sterilized deionized water, acidic electrolyzed water (AEW), or peroxyacetic acid (POAA), with agitation at 60 rpm using an agitator. After treatments, the fruit disks were immediately rinsed with D/E neutralizing broth (Difco Lab) for 5 s to neutralize residual sanitizer on the disk surfaces. The AEW was generated using an AEW generator (ROX-20TA, Hoshizaki, Nagoya, Japan) by electrolyzing 0.1% NaCl solution. The pH (< 2.7) and oxidation-reduction potential (ORP ≥ 1150 mV) of the AEW were measured with an AR15 pH and ORP meter (Accumet Research, Pittsburgh, Pa., U.S.A.) and the residual chlorine concentration (74 mg/L) of AEW was determined with an EPA approved chlorine colorometric test kit (Model PCT-DR, LaMotte Co., Chestertown, Md., U.S.A.). POAA (80 mg/L; Tsunami 100; Ecolab, St. Paul, Minn., U.S.A.) was prepared according to the manufacturer's instructions. The concentration of POAA was measured with a POAA test kit provided by the manufacturer. All treatment solutions were stored at 4 °C and used within 1 h.

Surface hydrophobicity. The surface hydrophobicity of the aluminum and fruit samples was measured using the sessile drop technique (Weirauch and others 1993). The water contact angle was measured directly inside the liquid at an equilibrium position θ_e with a G40 Krüss goniometer (Krüss USA, Matthews, N.C., U.S.A.) through a microscope. The water droplet was released onto the surface by means of microsyringe. The droplet was made as small as possible to minimize the effect of gravity on the contact angle. Contact angles were expressed in degrees.

Microbiological analysis

The bacteria washed off from the aluminum stub surfaces were diluted and enumerated on the sorbital-MacConkey agar (SMAC) (Difco Lab) supplemented with cefixime-tellurite (CT) (Oxiod Ltd., Basingstoke, Hampshire, U.K.). Each composite sample of fruit disks was combined with 50 mL of sterile 0.1% peptone solution

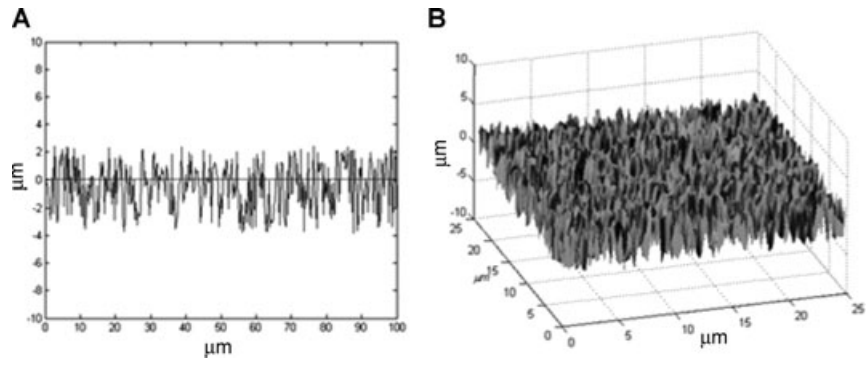

Figure 1 — The 2-D cross section of the surface roughness standard (A) and reconstructed CLSM 3-D image (B).

in a 400 mL sterile stomacher bag and blended with a Lab-Blender 400 (Cooke Lab. Products, Alexandria, Va., U.S.A.) for 4 min. The homogenate was filtered through sterile glass wool. A 100-μL sample of each filtrate and its appropriate dilutions were plated in triplicate in CT-SMAC agar. All CT- SMAC plates were incubated at 37 °C for 24 h. For each plate, 2 typical *E. coli* O157:H7 colonies were chosen and identified by an *E. coli* O157 Latex Test (Oxiod Inc., Ogdensburg, N.Y., U.S.A.).

Scanning electron microcopy

Freshly cut fruit disks were inoculated with a 5-strain cocktail described previously. The inoculated samples were air-dried for 2 h before treatment. All inoculated samples were divided into 2 groups: unwashed and washed samples (washed with sterilized deionized water for 5 min). After a treatment, the samples were fixed in 2.5 % (v/v) glutaraldehyde (E.M. grade) in 0.1 M Na·Cacodylate buffer (pH 7.2) for 4 h at refrigerator temperature and rinsed 3 times with 0.1 M Na·Cacodylate buffer every 10 min. The samples were postfixed in 1% (v/v) osmium tetroxide (OSO_4) solution for 90 min at room temperature in a biosafety hood in the dark and rinsed with 0.1 M Na·Cacodylate buffer every 10 min. Then the samples were dehydrated in a graded series of ethanol solutions (50%, 70%, 95%, and 100%) and dried in a CO_2 Critical-Point drier (Samdri-DVT-3D, Tousimis Research Corp., Rockville, Md., U.S.A.). The dry samples were mounted on aluminum stubs coated with a thin layer of gold-palladium by a Dest II TSC sputter coater and examined by environmental scanning electro microscopy (ESEM, Philips XL30 ESEM-FEG, FEI Co., Hillsboro, Oreg., U.S.A.).

Statistical analysis

All samples used for surface roughness measurements and enumerations of *E. coli* O157:H7 were prepared in triplicate. Data were analyzed using the general linear models procedure of the SAS

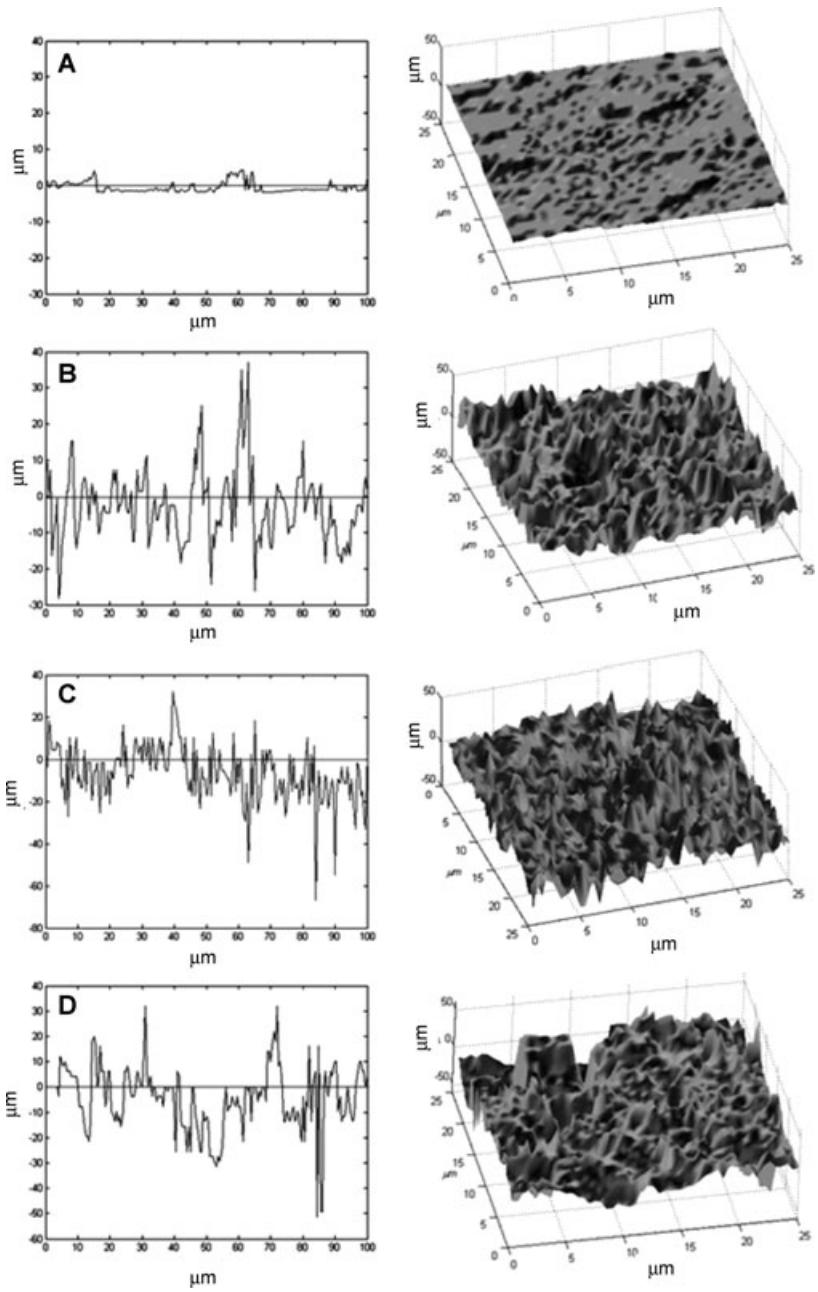

Figure 2—The reconstructed CLSM 3-D images and 2-D cross section of selected fruit surfaces. The 3-D images represent 1/8 of the whole image (100 × 100 × 50 μm) of a fruit surface and the 2-D cross section is from the whole image. (A) apple surface with $R_a = 1.43 \pm 0.13$ μm, (B) avocado surface with $R_a = 9.58 \pm 0.27$ μm, (C) orange surface with $R_a = 10.94 \pm 0.07$ μm, and (D) cantaloupe surface with $R_a = 14.18 \pm 0.25$ μm.

software program (SAS Inst., Inc., Cary, N.C., U.S.A.). Significant difference ($P \leq 0.05$) between mean values was determined using Fisher's LSD test.

Results and Discussion

Surface roughness determination

Figure 1A is a typical surface profile of a 2-D cross section of the nickel specimen obtained with CLSM. The 3-D image reconstructed from the 2-D layered images is shown in Figure 1B which is 1/8 of whole reconstructed image ($100 \times 100 \times 10$ μm). The R_a value of the specimen obtained using the CLSM method was 1.55 ± 0.04 μm, which was not significantly different ($P > 0.05$) from the R_a measured with the profilometer (1.52 ± 0.03 μm).

The surface topographic information of apple, avocado, orange, and cantaloupe given in 3-D images and surface profiles from 2-D CLSM measurements are illustrated in Figure 2. From the reconstructed 3-D images, the R_a of apple, avocado, orange, and cantaloupe samples were 1.43 ± 0.13, 9.58 ± 0.27, 10.94 ± 0.07, and 14.18 ± 0.25 μm, respectively. Among the 4 fruits, apples had the smoothest surface while the cantaloupes had the highest surface roughness value. These results were in agreement with visual observations. The 2-D cross sections of cantaloupe, orange, and avocado surfaces showed large valleys and peaks. The ESEM image (Figure 3) showed some huge nets and caverns on the surface of the cantaloupe which made it impossible to quantify surface roughness with current contact mode techniques. Although the ESEM image showed less rough topology on orange and avocado surfaces than those on the cantaloupe surfaces, the 3-D images or 2-D cross section indicated that there exist some valleys that were deeper than 10 μm, resulting in relatively high R_a values for these 2 fruits. The smooth apple surface in the ESEM image was also confirmed in the 3-D image and 2-D cross section with small height variations.

ESEM is a good tool for observation of fruit surfaces; however, it cannot provide quantitative roughness values. The CLSM makes it possible for extracting topographic information of fruit samples by focusing at different heights (along the z-axis) on the sample surfaces. The resolution in the z-direction is the most important in acquiring a series of 2-D layered images. The resolution in the z-direction depends on the numerical aperture of the lens, the degree to which the pinhole is open, and the wavelength of the laser light (Ding and Gunasekaran 1998). The minimum z-axial resolution and maximum observation depth achieved by CLSM used in this study was 50 nm and 200 μm, respectively. The scan area for CLSM measurement was 100×100 μm. In this 100 μm distance/length, the height change caused by the curvature of the 4 fruits was less than 3.6×10^{-2} μm, which is at least 2 magnitudes smaller compared to the surface roughness values obtained in this study. The effect of the curvature of the fruits on the measured R_a values can hence be ignored. A limitation of the CLSM in acquiring images is that the minimum distinguishable area in x–y plane is approximately 0.2×0.2 μm which determines the pix area on the 2-D images. In addition, care needs to be taken to avoid drifting of the sample due to water evaporation while acquiring 2-D images.

Influence of metal surface roughness on *E. coli* O157:H7 removal

From Table 1, it can be seen that the residual bacterial population and bacterial adhesion rate increase with the increase in surface roughness. A positive linear relationship ($R^2 = 0.96$) was found for the increase in residual bacterial population as a function of surface roughness, with a significant ($P < 0.05$) increase in residual bacterial population observed over the entire range of surface roughness evaluated, from 0.30 to 8.41 μm. A rough surface with a R_a larger than the dimension of *E. coli* O157:H7 (around 1 to 2 μm $\times$ 0.5 μm) improves protection for bacteria against mechanical removal, chemical injury, or both. As suggested by Characklis (1981), the increased bacterial adhesion on rough materials could be due to (1) an increase in surface area for adhesion and (2) protection against shear forces at the wall.

The surface of an aluminum stub ($R_a = 8.41$ μm) as well as the distribution of bacteria on it after a washing treatment is shown in Figure 4. The stub surface is full of grooves and crevices. Observation (Figure 4B) revealed that many cells remained on the stub surface but were concentrated in the crevices and along grooves. During a wash treatment, the cells inside a crevice may not be exposed to the sanitizer and cannot be reached by the shear forces produced by the washing solution (Korber and others 1997). The rough surface also provides more contact points to facilitate firm attachment of the cells (Holah and Thorpe 1990; Leclercq-Perlat and Lalande 1994).

The surface hydrophobicity of aluminum stubs expressed with water contact angles was also presented in Table 1. Although all the aluminum stub surfaces were hydrophobic, they can be divided

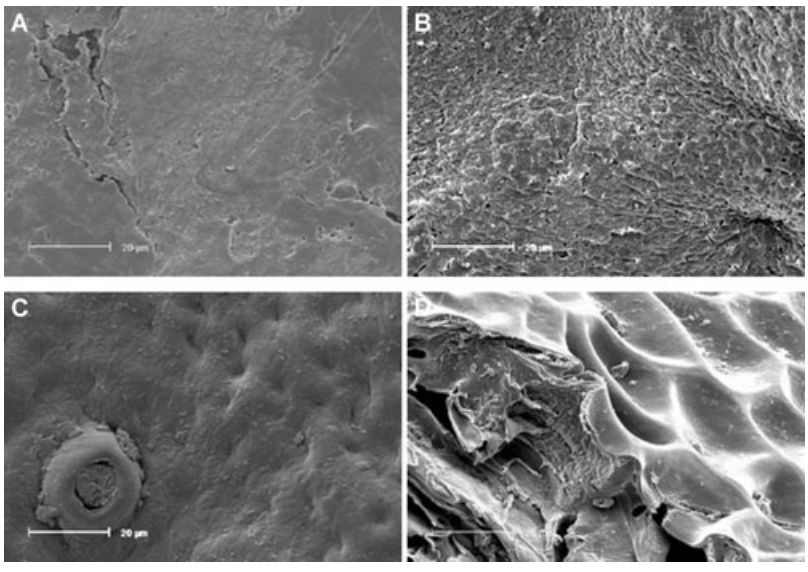

Figure 3—ESEM images of the surface topography of selected fruits at high magnification (×2400): (A) apple, (B) avocado, (C) orange, and (D) cantaloupe.

into 3 groups according to R_a values ($R_a < 1$ μm, 1 μm $< R_a <$ 5 μm, and $R_a > 5$ μm). When the $R_a < 1$ μm, the hydrophobicity remained almost unchanged with changes in surface roughness. When 1 μm $< R_a <$ 5 μm, surface hydrophobicity did change with changes in surface roughness, but the change was not significant. However, when the $R_a > 5$ μm, the surface hydrophobicity was significantly affected by changes in surface roughness ($P < 0.05$). A linear relationship was found between surface roughness and surface hydrophobicity ($Y = -3.3931X + 103.78$, $R^2 = 0.98$). For $R_a < 1$ μm and 1 μm $< R_a <$ 5 μm, an increase in surface roughness resulted in a significant increase in bacteria retention, but an insignificant change in surface hydrophobicity (Table 1), which indicates that the surface roughness played a more important role in bacteria removal from the surface than that of the surface hydrophobicity.

Table 1—*E. coli* O157:H7 population on aluminum stubs after sonication in water for 2 min.

Surface roughness (R_a, μm)	Surface hydrophobicity (θ_e)	Residual bacteria population (log CFU/mL)	Adhesion rate[a]
0.30 ± 0.08	102.50 ± 3.04	0.72 ± 0.17	111 ± 5
0.71 ± 0.22	101.00 ± 2.00	0.89 ± 0.13	137 ± 2
3.36 ± 0.73	91.33 ± 4.16	1.41 ± 0.23	218 ± 7
4.18 ± 0.35	92.00 ± 6.24	2.14 ± 0.28	331 ± 8
8.41 ± 2.54	74.50 ± 0.87	2.34 ± 0.08	360 ± 3

[a]Adhesion rate was defined as the ratio 1000 × (residual bacteria counts/initial bacteria counts).
Lécrigny-Nolf and others (2000).

Influence of fruit surface topography on *E. coli* O157:H7 removal

The adhesion rates of *E. coli* O157:H7 cells increased with R_a values of the 4 types of fruits treated with sterilized deionized water, AEW, and POAA (Table 2). A positive linear relationship between R_a values and residual bacterial populations was obtained for water ($R^2 = 0.93$), AEW ($R^2 = 0.77$), and POAA ($R^2 = 0.95$) treatments. From the results of the washing tests, as well as from the ESEM images (Figure 3) and reconstructed 3-D images (Figure 2), 3 types of fruit surfaces can be identified: (1) smooth surfaces with few peaks

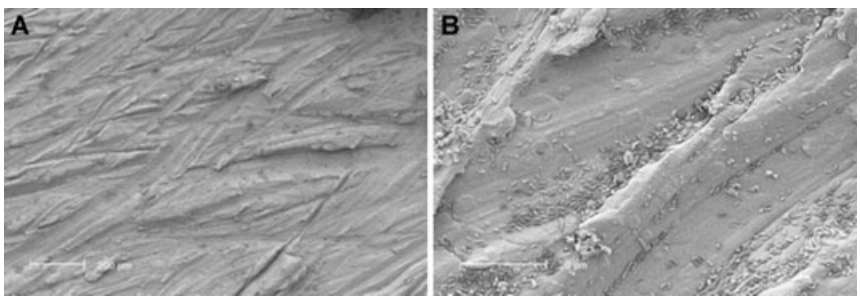

Figure 4—ESEM images of *E. coli* O157:H7 cells retained on the surface of an aluminum stub ($R_a = 8.41 \pm 2.54$ μm) after a sonication treatment. (A) the surface of an aluminum stub at low magnification (×300) and (B) the surface of an aluminum stub at high magnification (×2400).

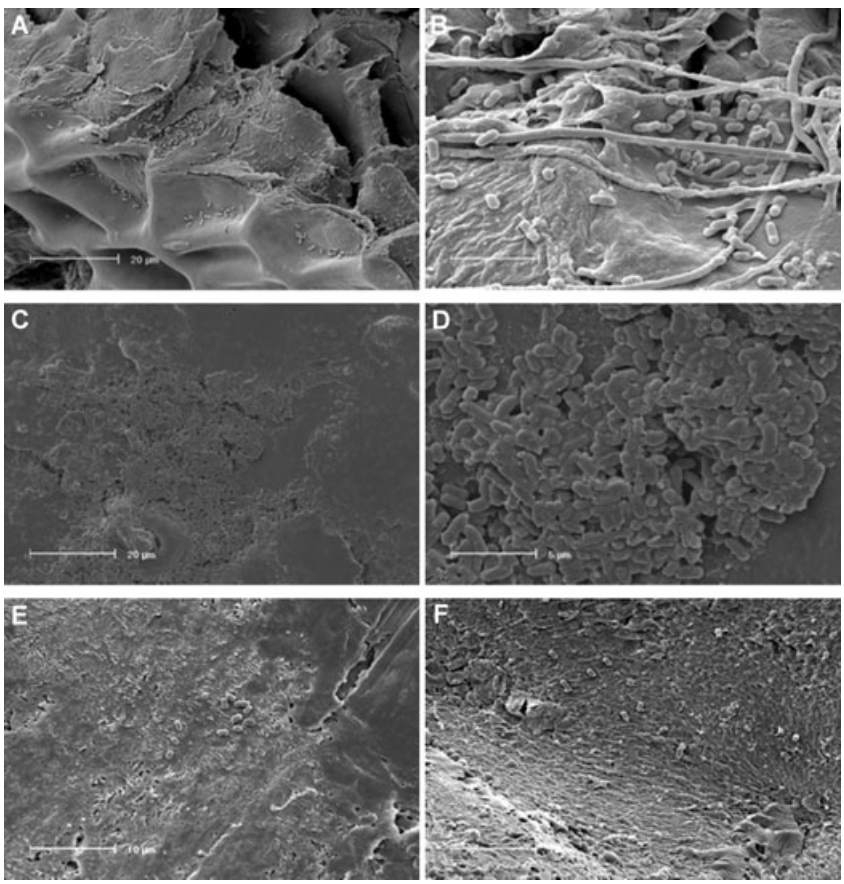

Figure 5—ESEM images of *E. coli* O157:H7 cells attached to fruit surfaces: (A) cantaloupe surface inoculated with *E. coli* cells and dried for 2 h at room temperature and treated by a water wash (×2400), (B) the same cantaloupe sample incubated for 22 h at 37 °C (×9600), (C) orange surface inoculated with *E. coli* cells dried for 2 h at room temperature and incubated for 22 h at 37 °C (×2400), (D) the same orange surface at a higher magnification (×9600), (E) apple surface inoculated with *E. coli* cells dried for 2 h at room temperature and incubated for 22 h at 37 °C (×4800), and (F) avocado surface inoculated with *E. coli* cells dried for 2 h at room temperature and incubated for 22 h at 37 °C (×4800).

Table 2—*E. coli* O157:H7 population on fresh fruit surfaces after a 5-min washing treatment.

Sample and surface roughness (R_a, μm)	Surface hydrophobicity (θ_e)	Residual bacteria population (log CFU/cm^2)			Adhesion rate[a]		
		Water	AEW	POAA	Water	AEW	POAA
Apple (1.43 ± 0.13)	77.27 ± 4.57	2.61 ± 0.20	2.60 ± 1.22	2.23 ± 0.71	490 ± 6	480 ± 21	420± 16
Avocado (9.58 ± 0.27)	78.23 ± 8.37	3.99 ± 0.33	3.57 ± 0.91	3.79 ± 0.03	620 ± 6	560 ± 15	590 ± 0
Orange (10.94 ± 0.07)	56.33 ± 5.16	5.19 ± 0.19	3.87 ± 0.67	4.60 ± 0.17	930 ± 2	690 ± 13	820 ± 2
Cantaloupe (14.18 ± 0.25)	47.20 ± 18.52	6.03 ± 0.29	5.98 ± 0.03	5.81 ± 0.49	920 ± 6	890 ± 6	910 ± 1

[a]Adhesion rate: see Table 1.

or grooves (apples) where attached bacteria were exposed to mechanical forces of the washing solutions, (2) rough surfaces with a few deep valleys (oranges or avocados) where attached bacteria were to some extent exposed to mechanical forces from washing, and (3) very rough surfaces with huge cavities and wide valleys (cantaloupes), where attached bacteria were well protected by surface features and hence had high resistance to mechanical forces during a washing operation.

The surface hydrophobicity of fruits has relatively large standard deviations (Table 2), probably due to the effect of fruit ripeness and locations of a droplet of water dropped during the contact angle measurement. There was a quadratic relationship ($Y = -0.3759X^2 + 3.3283X + 3.65$, $R^2 = 0.82$) between surface roughness and surface hydrophobicity of fruits. The residual bacterial populations acquired from apple and avocado surfaces after washing treatments are different, as shown in Table 2. Since apples and avocados had similar surface hydrophobicity but different R_a, it may indicate again that the surface roughness is a more important factor relating to bacteria removal from the surface than the surface hydrophobicity. Once a bacterium was attached to a surface, the irregularity or defects on the surface could provide protection against a washing treatment. This is evidenced by the washing tests conducted by Sapers and Simmons (1998) who used chlorine to wash cantaloupes with a rough surface and found that it was ineffective, while washing apples with a commercial cleaner resulted in 2.86 log reduction in the viable count of *E. coli* O157:H7 (Kenney and Beuchat 2002).

Figure 5A shows that the *E. coli* O157:H7 cells inoculated on the cantaloupe surface for 2 h were entrapped and retained in the valleys or cavities after a water wash. These entrapped cells become firmly attached to the surface after a period of time. This is shown in Figure 5B where the entrapped cells grew after 22 h incubation at 37 °C. Although the residual bacterial population on the apple, avocado, and orange surfaces was countable right after water or sanitizer treatments (Table 2), the bacteria were hardly observed on the ESEM images (images not shown). The bacteria inoculated on fruit surfaces and dried for 2 h at room temperature were still in the reversible adsorption stage, the 1st step in Fletcher and Floodgate's (1973) theory on bacterial attachment. At this stage, bacteria are attracted by relatively weak van der Waal interactions to the substratum, but tend to be repelled by electrostatic interactions. Thus, they can be easily washed off the surface during the preparation of the specimen for ESEM observation, as the samples are washed 11 times with the dehydration solutions. The bacteria inoculated on the apple, avocado, and orange surfaces, dried for 2 h at room temperature, and followed by incubation for 22 h at 37 °C are shown in Figure 5C, 5E, and 5F, respectively. The remaining bacteria on the fruit surfaces may grow into colonies (Figure 5D) after 22 h of incubation at 37 °C, which increased the resistance to wash treatments.

Conclusions

The confocal scanning laser microscopy (CLSM) technique was used to develop a new noncontact quantitative method to assess the surface roughness of fresh fruits. The method developed was validated with a standard metal stub. It provides a feasible tool to study the contribution of surface topography of fruits and vegetables to the attachment and removal of microorganisms on food surfaces. The correlation between surface roughness and surface hydrophobicity can be described with a linear equation for metal stubs and a quadratic equation for selected fruits. The effects of surface roughness on microbial reduction were quantified, for both metal surfaces and fruit surfaces, by a linear relationship, indicating that an increase in surface roughness would introduce protection to microbes entrapped on fruit surfaces resulting in reduced washing efficacy. The information reported in this study will be helpful to the fresh produce industry in its effort to enhance microbial safety in produce production.

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