Produce Surface Characteristics Affect Product Quality and Safety

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Abstract

The surface characteristics of fresh-cut fruits and vegetables vary greatly among produce types, varieties, maturities and growing conditions. Studies have shown that surface conditions of produce significantly affected adherence, attachment, and reduction of pathogens during wash process. Using confocal laser scanning microscopy, the surface roughness of apples, avocadoes, and cantaloupes was estimated and compared with the reduction rate of pathogen. It was found that the surface roughness played an important role in pathogen reduction rate during produce wash with the lowest roughness/highest pathogen reduction rate obtained on apples and the highest roughness/lowest pathogen reduction rate obtained on cantaloupes.

INTRODUCTION

Many studies have examined the efficacy of sanitizers on microbial inactivation on fresh and fresh-cut produce. An important and often obvious observation is that the same concentration of a sanitizer causes different bacterial population reduction when washing different produce (Wang, 2006; Wang et al., 2006). It has also long been observed that the resistance of bacteria to a sanitizer in a cell suspension is much smaller than that of cells attached to a surface (Frank, 2001). Since most produce disinfection methods are surface treatments, the differences in fresh produce surface conditions may play an important role in microbial reduction. Babic et al. (1996) and Seo and Frank (1999) observed that bacteria are often attached to cracks in cuticle, broken trichomes and stomata. Han et al. (2000) reported that *Escherichia coli* O157:H7 more preferentially attached to coarse, porous, or injured surfaces than uninjured surfaces of green peppers. Liao and Sapers (2000) observed a higher attachment rate of *Salmonella chester* to injured tissue of apples than to the intact skin. A good understanding of the role played by produce surface conditions on bacterial population reduction will help to better improve the washing efficacy.

Produce surface roughness is an important parameter for describing surface characteristics. The average roughness (R_a), an arithmetic average of the absolute values of the surface height deviations measured from the mean plane, has been widely used to describe the surface topology of a solid substance in the material science field (Verran and Boyd, 2001). However, determining surface properties of fruits and vegetables has been difficult, because most direct contact methods commonly used by material scientists cannot be directly applied to the soft produce surface due to errors introduced by surface deformation. To address this issue, several indirect methods, e.g., glistening points method and fractal image texture analysis, have been tested to describe surface roughness of foods. The glistening points method estimates surface roughness indirectly from light reflection signals (Quevedo et al., 2004) while the fractal image texture analysis relies on an image processing method (Quevedo et al., 2002; Pedreschi et al., 2000). Both methods are based on 2-D information obtained on the subject and the 3-D information is lacking.

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Our earlier studies showed that confocal laser scanning microscopy (CLSM) can be used to take a series of 2-D layered images by optical slicing the surface by focusing at different heights (among the z-axis). Roughness (R_a) of the surface can be assessed by reconstructing a series of 2-D layered images into 3-D images (Ding and Gunasekaran, 1998). For surface observation purpose, the Syncroscopy AutoMontage software has been used to process images taken by different methods to obtain high resolution 3D surface images for biological materials (Twon, 1997).

The main objectives of this study were to examine the surface topography with CLSM and to evaluate the effect of water wash on the reduction on *E. coli* O157:H7 as influenced by the surface roughness of different fruits.

MATERIALS AND METHODS

Optical Image Acquisition Using a Syncroscopy Auto-montage System

Golden Delicious apples (*Malus domestica* Borkh.), avocadoes (*Persea americana* Mill.), and cantaloupes (*Cucumis melo* L. var. *reticulatus*) were purchased from a wholesale market and used immediately. Samples were first taken with a cork borer (20 mm diameter) and then cut into 10 mm discs with a sharp knife.

The surfaces of apples, avocadoes, and cantaloupes at 1x, 10x and 100x magnifications were photographed using a high resolution 3CCD JVC KY-75U digital camera attached to a Leica MZ 16 stereomicroscope (Leica Microsystems Inc. Bannockburn, IL, USA). A series of 2D images at different depths of field were acquired and converted into 3D images via a Syncroscopy Auto-Montage System (Frederick, MD, USA).

Surface Roughness Measurement Using Confocal Laser Scanning Microscopy

Confocal laser scanning microscopy (WITec Instruments Corp., Savoy, IL, USA) was used to take a series of 2-D images (100 µm x 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 avocado and cantaloupe. The observation depth was 6.4 µm for apple and 200 µm for orange, avocado and cantaloupe, respectively. The image layers were scanned from top to bottom and from left to right with a total of 128 adjacent planes (images) acquired for each sample. From these 2-D images, 3-D images of the samples were constructed.

The topographic information for fruit surfaces was obtained and expressed as the statistical parameter R_a: the arithmetic average of the absolute values of the surface height deviations measured from the mean plane $R_a = \frac{1}{N}\sum_x\sum_y Z(x,y)$, where *Z(x, y)* is the distance from each point to the mean plane $Z(x,y) = h(x,y) - M$, *M* is the height of the mean plane $M = \sum_x\sum_y h(x,y)$ and *N* is the total number of points on each image.

E. coli O157:H7 Inoculation, Sample Washing and *E. coli* O157:H7 Enumeration

A five-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 three times to tryptic soy broth (pH 7.3, Difco Lab, Detroit, MI, USA) by loop inoculation at successive 24 h intervals and incubated at 37°C. Twenty-four-hour bacterial cells were harvested by centrifugation (10,000×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 five cultures were mixed to obtain an inoculum containing approximately 10 log cfu/ml *E. coli* O157:H7.

Golden delicious apples, avocadoes and cantaloupes were sanitized with UV light for 20 min to inactivate naturally occurring microorganisms on the fruit surfaces. A sterilized brass cork-borer (No.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 (3-4 mm in thickness). The disks were prepared from different areas of the same fruit. After preparation, disks were pooled together, and five disks from the pool were randomly selected and used as a composite sample. Fruit disks were spot inoculated with 20 µl of the five-strain cocktail of *E. coli* O157:H7 (8.3 log cfu/ml). To facilitate drying, the 20 µl was deposited in 10 small drops on fruit disk surfaces. All inoculated samples were air dried for 2 h in a laminar flow biological hood. Inoculated fruit disks were washed for 5 min with 50 ml of sterilized de-ionized water. *E. coli* O157:H7 populations before and after water wash were then enumerated. Each composite sample of fruit disks was combined with 50 ml of sterile 0.1% peptone solution in a 400 ml sterile stomacher bag (Fisher Scientific Inc., Pittsburgh, PA, USA) and was blended with a Lab-Blender 400 (Cooke Laboratory Products, Alexandria, VA, USA) 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 on cefixime and tellurite (CT) - Sorbitol MacConkey agar. All plates were incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

The surface topography of apple, avocado, and cantaloupe at 1x, 10x, and 100x magnifications obtained by the Syncroscopy Auto-Montage System are shown in Figures 1-3. Among all three types of fruit surfaces, apple surface appears to be the smoothest, followed by avocado, while cantaloupe rind surface has the roughest appearance.

Surface roughness measurements obtained with a confocal laser scanning miscopy are displayed in Figure 4A. Apple surface had a lowest surface roughness value (1.43 µm), followed by avocado (9.58 µm), while cantaloupe had the highest roughness reading (14.18 µm). This result agrees well with the observations made under the Syncroscopy Auto-Montage System (Fig. 1-3). Hershko et al. (1998) used atomic force microscopy (AFM) to estimate the surface area of garlic and onion skins. They obtained a roughness factor (ratio between apparent and measured surfaces) of 1.11 to 1.15 for untreated and chloroform-treated onion skins. Using digital images, Quevedo et al. (2002) calculated the fractal dimension values of the surfaces of apples, potatoes, and pumpkins that were 2.28-2.30, 2.30-2.35 and 2.35-2.45, respectively. Quevedo and Aguilera (2004) reported a σ of 25.7 ± 2.6 µm for apples, where σ is the amplitude of the root-mean-square roughness obtained with the glistening points method under the assumption of a Gaussian distribution of heights over a rough surface. Since few studies have been conducted to quantify the surface roughness of fruits and vegetables, data are not available to directly compare with the R_a readings obtained in this study.

The log reduction of *E. coli* O157:H7 populations on the three fruits after washing is shown in Figure 4B. A 5-min wash with de-ionized water resulted in the highest reduction rate on *E. coli* O157:H7 populations on apples, followed by avocadoes, and the lowest reduction rate on cantaloupes. A negative correlation was found between the reduction rates in *E. coli* O157:H7 populations caused by water wash and the roughness values of the fruits tested. Although treating the fruit surfaces with sanitizers, e.g., acidified electrolyzed water and peroxyacetic acid increased the reduction rates of *E. coli* O157:H7 on all fruits tested, there was a higher *E. coli* O157:H7 reduction rate found on apple surfaces than on avocadoes, and cantaloupes (data not shown). A few previous studies have documented the efficacy of sanitizers on reduction of microorganisms on different produce surfaces. Kim et al. (2006) reported that chlorine was less effective in killing *Enterobacter sakazakii* on lettuce than on apples or tomatoes. Michaels et al. (2003) washed different fruits and vegetables with water and found that the log reduction of natural flora was in the order of apple > grape > carrot > strawberries. No report has been found in the literature correlating microbial reduction during sanitation to surface roughness of the produce. Figures 4A and B also demonstrate that fruit surface roughness is not the only factor involved in bacterial reduction as the bacterial reduction rate on avocado is only slightly lower than that of apples even though the surface roughness index is much larger than apples. The high bacterial reduction rate obtained on avocado suggests that the physical and chemical composition of avocado skins may have played a significant role in the removal of bacteria during washing.

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Figures

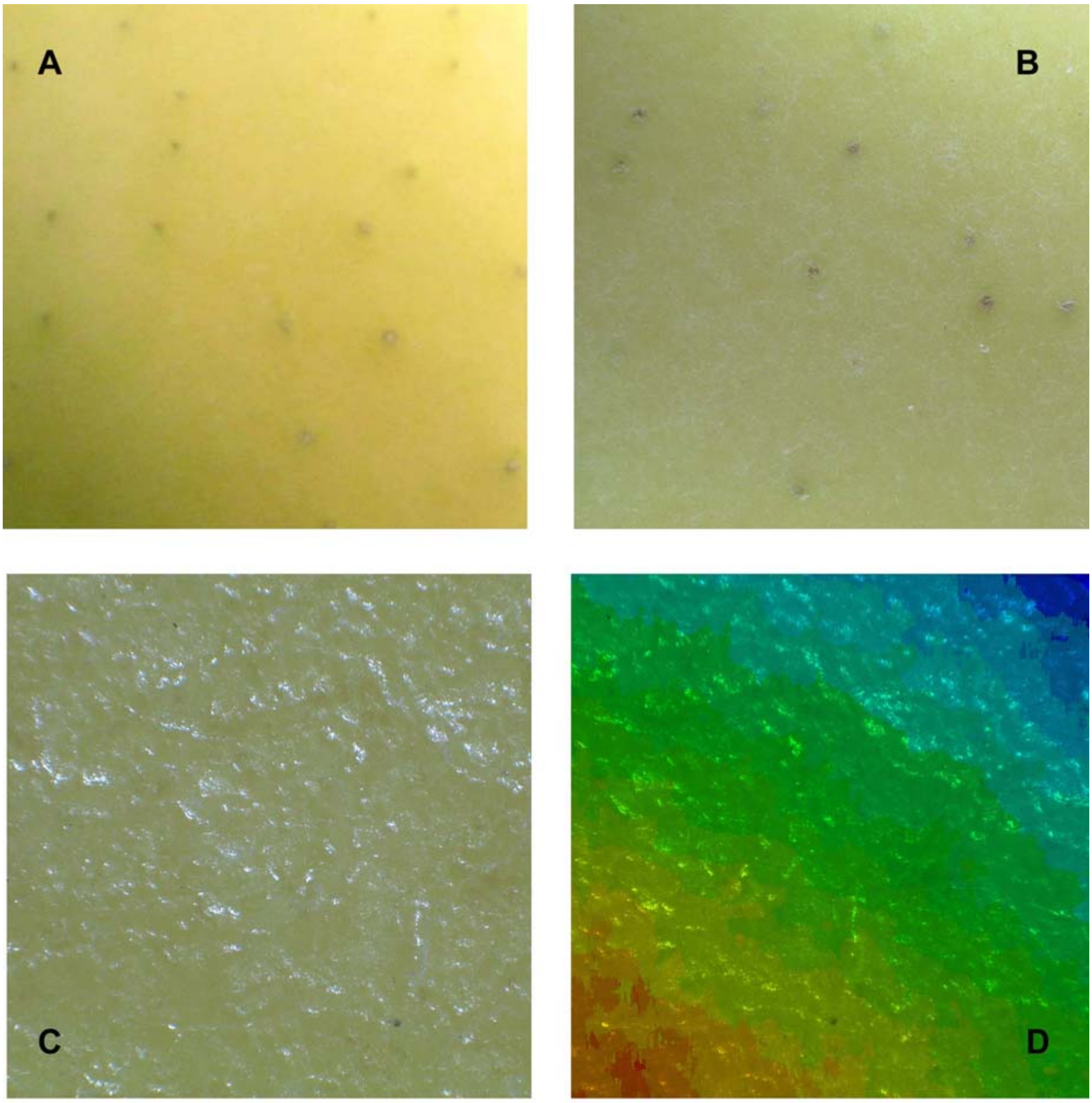

Fig. 1. Images of apple surface acquired at magnifications of (A) 1x, (B) 10x, (C) 100x, and (D) 100x with color relief.

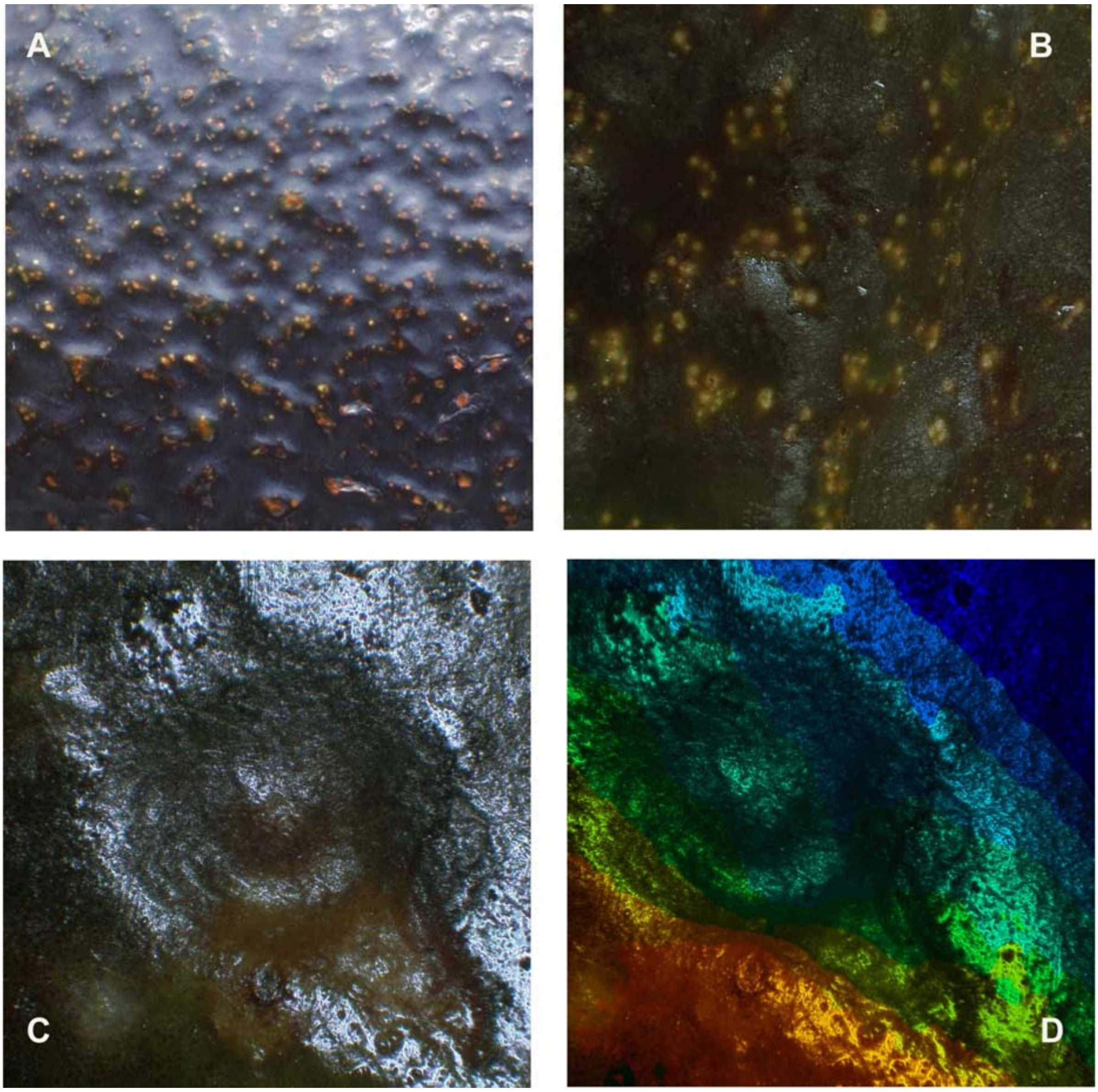

Fig. 2. Images of avocado surface acquired at magnifications of (A) 1x, (B) 10x, (C) 100x, and (D) 100x with color relief.

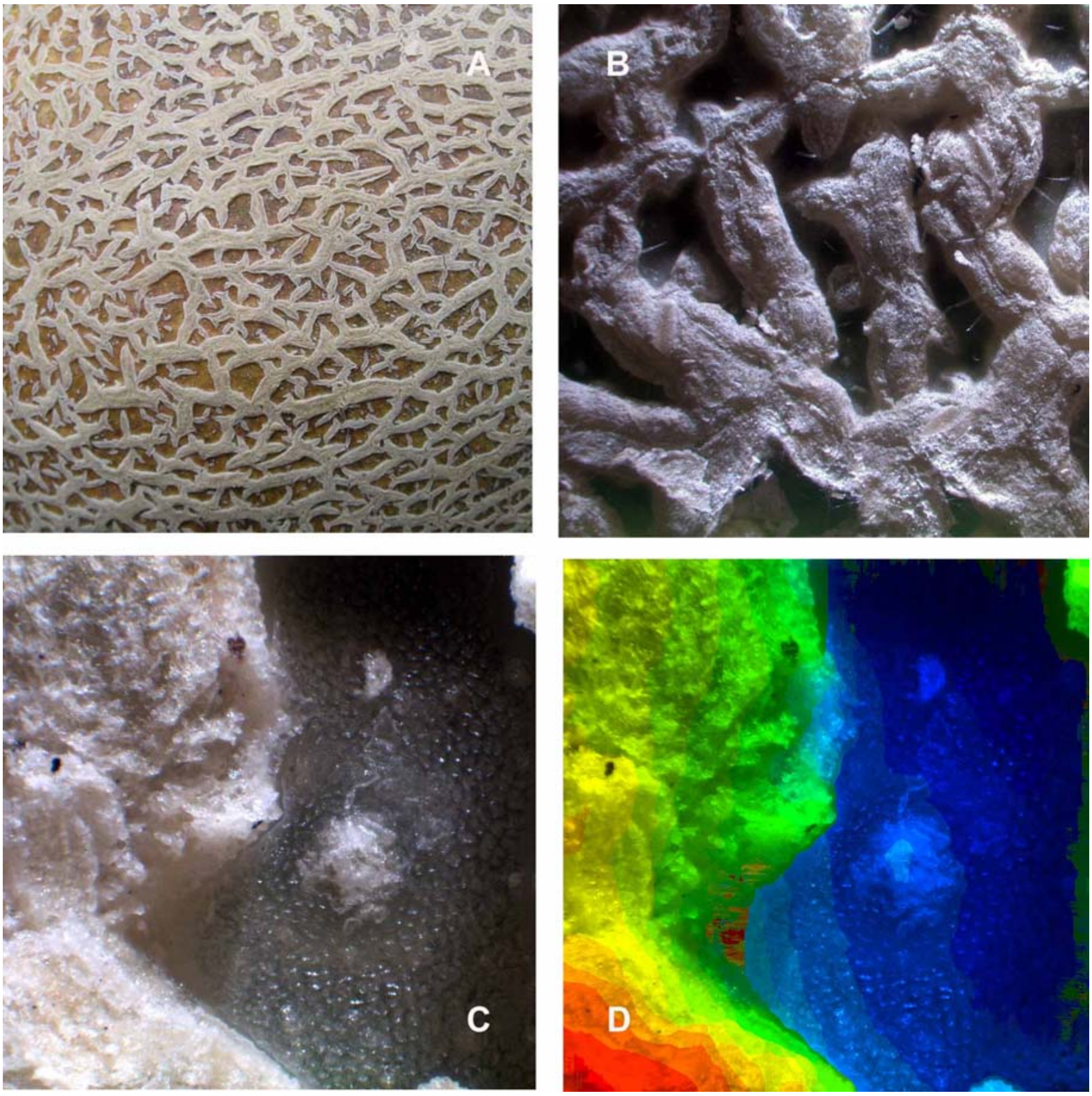

Fig. 3. Images of cantaloupe surface acquired at magnifications of (A) 1x, (B) 10x, (C) 100x, and (D) 100x with color relief.

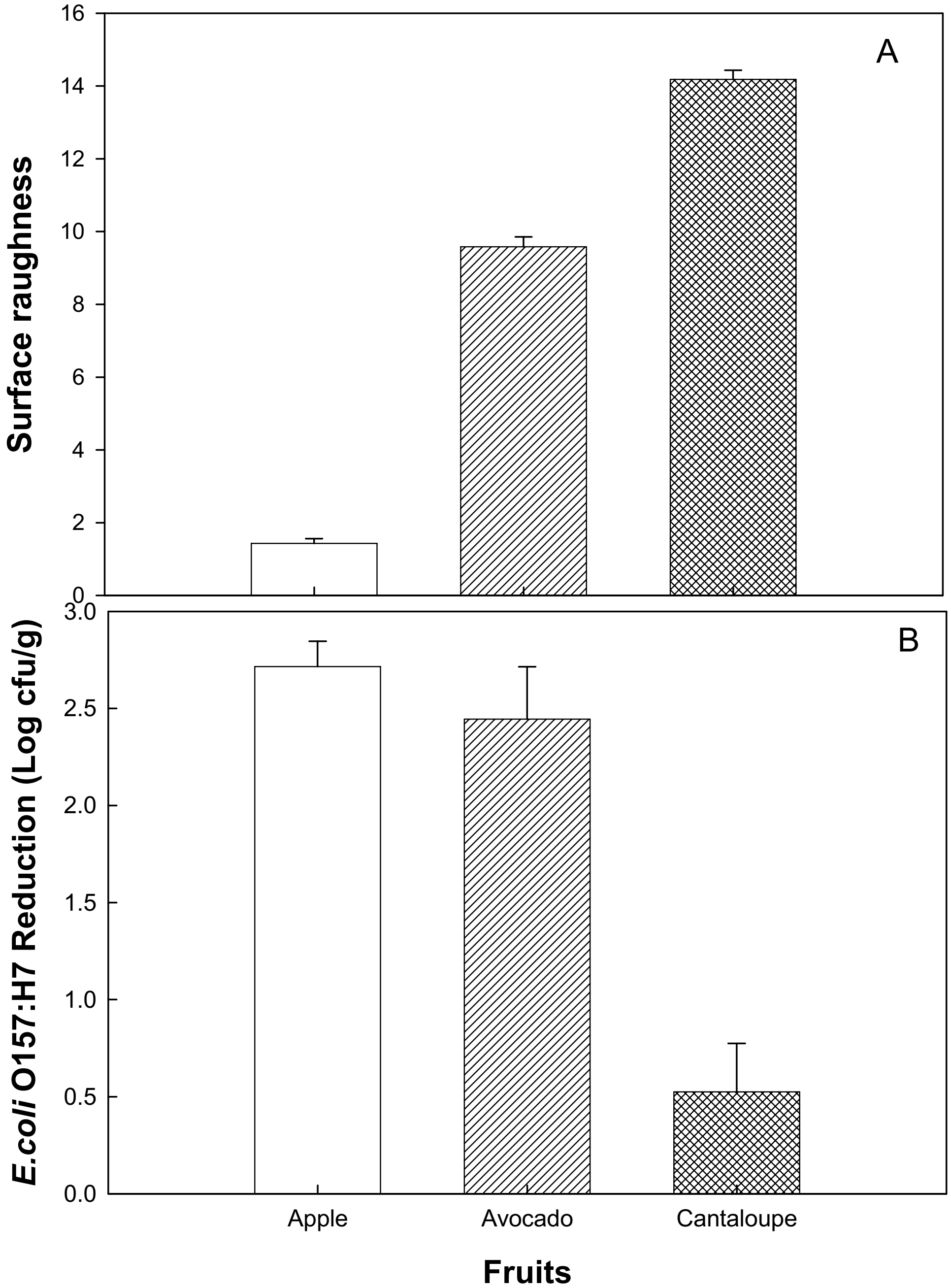

Fig. 4. Surface roughness of apples, avocadoes, and cantaloupes (A), and the reduction rate on *E. coli* O157:H7 populations on apples, avocadoes and cantaloupes after washing with water for 5 min. Data represent means ± standard deviation of 3 replications.