Effect of Surface Properties on Colloid Retention on Natural and Surrogate Produce Surfaces

Volha Lazouskaya, Taozhu Sun, Li Liu, Gang Wang, and Yan Jin

Abstract: Bacterial contamination of fresh produce is a growing concern in food industry. Pathogenic bacteria can attach to and colonize the surfaces of fresh produce and cause disease outbreaks among consumers. Surface properties of both bacteria and produce affect bacterial contamination; however, the effects of produce roughness, topography, and hydrophobicity on bacterial retention are still poorly understood. In this work, we used spherical polystyrene colloids as bacterial surrogates to investigate colloid retention on and removal (by rinsing) from fresh produce surfaces including tomato, orange, apple, lettuce, spinach, and cantaloupe, and from surrogate produce surface Sharklet (a micro-patterned polymer). All investigated surfaces were characterized in terms of surface roughness and hydrophobicity (including contact angle and water retention area measurements). The results showed that there was no single parameter that dominated colloid retention on fresh produce, yet strong connection was found between colloid retention and water retention and distribution on all the surfaces investigated except apple. Rinsing was generally not efficient in removing colloids from produce surfaces, which suggests the need to modify current cleaning procedures and to develop novel contamination prevention strategies. This work offers a physicochemical approach to a food safety problem and improves understanding of mechanisms leading to produce contamination.

Keywords: colloids, food safety, fruits and vegetables, image processing, physicochemical properties

Practical Application: This study provides mechanistic understanding of processes leading to colloid retention on fresh produce. This knowledge can improve bacterial contamination prevention and cleaning practices. Also, it can be potentially applied to surface modification of sensitive produce to reduce contamination.

Introduction

Bacterial contamination of fresh produce is an important safety concern in food industry and for consumers (Berger and others 2010; Olaimat and Holley 2012). It has been reported that surfaces of certain produce types or cultivars retain higher numbers of bacteria than others (for example, Patel and Sharma 2010; Erickson 2012). Also, the efficacy of sanitizing techniques differs among produce types pointing at the importance of produce surface properties in bacterial contamination and de-contamination (Wang and others 2009; Wang and others 2012). A number of produce physicochemical properties affecting contamination have been discussed in the literature including surface roughness, topography, and hydrophobicity (Wang and others 2009; Fernandes and others 2014), yet the governing properties and underlying mechanisms of bacterial retention remain poorly understood (Zhang and others 2014).

Surface topography is defined as specific arrangement of physical features on a surface (Hsu and others 2013). In metrology, surface topography is characterized by 3 components: form, waviness, and roughness. These components correspond to different scales, at which topography is considered, and can be separated by mathematical filtering of topography data (Wennerberg and Albrektsson 2000; Crawford and others 2012). Roughness is a small-scale component of topography (Whitehead and Verran 2006; Hsu and others 2013), which is quantified and described in terms of roughness parameters (Whitehead and Verran 2006). In this work, we refer to topography as a qualitative surface characteristic at a larger scale (as arrangement of features sized tens of microns and larger) and to roughness as a quantitative micro-scale surface characteristic.

Effect of surface roughness on bacterial retention on various surfaces has received considerable attention (Crawford and others 2012); however, the results have not been always consistent (Flint and others 2000; Crawford and others 2012). Very few studies investigated effects of surface roughness on bacterial retention on fresh produce (Wang and others 2007; Wang and others 2009; Fernandes and others 2014). Roughness is commonly characterized using amplitude parameters such as arithmetic mean roughness (R_a) or root-mean-square roughness (R_q). It has been suggested that bacteria attach less below certain R_a values, for example, $R_a \leq 0.8$ μm (Flint and others 2000; Hsu and others 2013), and there may be a range of roughness values more favorable for bacterial trapping and retention (Hou and others 2011). However, some researchers have indicated that parameters R_a and R_q may not be sufficient for complete roughness characterization (Hou and others 2011) and that additional roughness parameters should be employed (Wennerberg and Albrektsson 2000; Crawford and others 2012). It has been also suggested that other surface properties in addition to roughness should be considered, such as topography (Hou and others 2011; Hsu and others 2013) and hydrophobicity (Flint and others 2000).

Indeed, surface topography of fresh produce has been linked to bacterial retention (Wang and others 2009, 2012; Kroupitski

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and others 2011). Increased bacterial retention was observed in the vein regions of lettuce, cilantro, and other leaves (Brandl and Mandrell 2002; Brandl 2006; Kroupitski and others 2011), which could be related to increased water availability in the vein regions (for example, Brandl 2006). Other preferred surface features and locations of bacterial retention on fresh produce and leaves included entrapment in depressions in the cuticle, epidermal cell wall junctions, trichomes, stomata, and so on. (Iturriaga and others 2003; Baldotto and Olivares 2008).

Hydrophobicity of fresh produce is crucial for bacterial retention as it affects produce-bacteria interactions, water movement, and bacterial distribution on produce surface (Wang and others 2009, 2012). Nevertheless, the effect of produce hydrophobicity on bacterial retention has not received much attention (Wang and others 2012). Hydrophobicity of fresh produce is mostly determined by the presence of epicuticular wax (Lindow and Brandl 2003; Lu and others 2015) and affected by surface roughness (Lu and others 2015).

Although importance of fresh produce properties in bacterial retention has been acknowledged in numerous studies, few systematic studies have been conducted to identify governing parameters and mechanisms in bacterial retention (Berger and others 2010; Zhang and others 2014).

There are a number of processes occurring on the surfaces of produce upon their contact with water phase during rain, irrigation, wash, and so on, and leading to potential contamination. Such processes may include submersion of produce into water, residual water retention and distribution on produce surface, movements of receding and advancing contact lines, and evaporation. These processes are greatly influenced by the produce surface properties such as topography, roughness, and hydrophobicity. Both bacterial retention and removal are affected by these processes: particle movement with advancing and receding contact lines has been extensively discussed in the literature (for example, Aramrak and others 2013; Lazouskaya and others 2013; Snoeijer and Andreotti 2013). However, such advanced understanding has not been applied to provide insights to issues related to produce contamination.

We conducted a comprehensive investigation on the role of physicochemical properties of produce such as surface roughness, topography, and hydrophobicity in colloid retention, distribution, and removal for 6 produce types and one control polymeric substrate Sharklet whose surface features are known to inhibit bacterial retention (Schumacher and others 2007; Mann and others 2014). In this work, we used colloidal microspheres to represent bacteria in order to exclude biological effects of bacteria and to focus on the properties of produce. Colloids as bacterial surrogates have been previously used in the studies with lettuce plants (Solomon and Matthews 2005) and taro leaves (Ma and others 2011).

Materials and Methods

Sample preparation

Extra-large tomatoes, organic navel oranges, organic Gala apples, iceberg lettuce, organic baby spinach, and cantaloupe were purchased from local supermarkets (Acme, Newark, DE and Trader Joe's, Wilmington, Del., U.S.A.) and stored at 4 °C. Artificial micro-patterned polymer surface Sharklet was purchased from Sharklet^{TM} Technologies, Inc. (Aurora, Colo., U.S.A.). The abaxial and adaxial sides of spinach and lettuce leaves were investigated separately and are referred to as "spinach abaxial," "lettuce abaxial," "spinach adaxial," and "lettuce adaxial." Two visibly different areas of cantaloupe surface are referred to as cantaloupe top and valley. Only fresh produce free of visible defects was used for sample preparation. Produce was brought to room temperature prior to sample preparation, washed gently with tap water, then rinsed well with deionized (DI) water, and dried in a fume hood. Produce surfaces were cut into approximately 2 × 2 cm squares using a stainless steel knife, and Sharklet sheets were cut with scissors. To prevent destroying the epidermal tissue of lettuce and spinach, these samples were torn by hand. The samples were then used right away for colloid retention (batch) experiments or surface characterization.

FluoSpheres® carboxylate-modified red microspheres with diameters of 1.0 μm (F8821, Molecular Probes®, Thermo Fisher Scientific, Waltham, Mass., U.S.A.) were used. The colloids were dispersed in 0.3 mM NaCl solution at a concentration of 10 ppm. The same stock colloid suspension was used in all experiments.

Batch experiments

Fifteen milliliters of 10 ppm colloid suspension were added to 50-mL disposable centrifuge tubes. Produce samples were placed into the colloid suspension with the skin or surface of interest facing the tube bottom. The tubes were put on a shaker at 140 rpm for 1 h. Produce samples were oriented vertically, pulled out of colloid suspension in a fast motion (at velocity U approximately 0.1 m/s) with tweezers, and placed on Petri dishes for drying and imaging for retained colloids. The relatively high velocity of sample withdrawal was chosen based on the Landau–Levich–Derjaguin (LLD) theory (for example, Quéré 1999) in order to have a microscopic water film on produce surface and thus minimize contact line effects on retained colloids. Once all the samples were visually dry, sample surfaces and retained colloids were imaged with a confocal microscope. Major processes that occurred during this experiment are shown in Figure 1(Schematic A).

In addition to colloid retention, colloid removal was analyzed in "rinsing" experiments. Rinsing experiments started with the same procedure as described above, then the samples, taken out of colloid suspension, were dipped into colloid-free 0.3 mM NaCl solution for 10 min. The tubes were hand-shaken gently and then the samples were taken out in the same manner as above (Figure 1, Schematic B). This procedure represents a washing practice in which the produce is soaked in water and then removed.

To minimize the effects of nonuniform residual water retention on sample surfaces (Figure 1, A-4), the samples were imaged in the sample center because maximum water pooling was observed at the sample edges.

Both colloid retention and rinsing experiments were performed in duplicates or triplicates.

Confocal microscopy and image analysis

Colloid retention on sample surfaces was imaged using an upright laser scanning confocal microscope Zeiss 780 (Carl Zeiss, Inc., Jena, Germany) equipped with a 20× air lens (EC Epiplan-Apochromat 20×, NA 0.6). Depending on surface heterogeneity and colloid retention pattern, 4 to 8 locations per sample were imaged, and number of images per sample type ranged from 8 (for Sharklet) to 34 (for apple). Two channels were used for imaging including a fluorescent channel for imaging red fluorescent colloids (561 nm laser line) and a reflection channel for imaging surface topography (633 nm laser line). The images were taken as z-stack images with sizes of 425 × 425 μm (1024 × 1024 pixels) and an optical slice interval of 1.64 μm. Maximum intensity projections were generated from z-stacks and used in image analysis.

Colloid retention in confocal images was quantified with image analysis software Volocity® (PerkinElmer Inc., Waltham, Mass., U.S.A.). Colloids were identified and counted automatically using a defined fluorescence intensity threshold. Colloid aggregates (2 or more colloids aggregated together) were identified by colloid image area, for example, as colloids with areas larger than 1.5 μm^2.

Surface roughness

Surface roughness was characterized using the arithmetic mean roughness parameter S_a calculated for the surface area (surface area analog of line roughness R_a). S_a is calculated as (Stout and others 1993):

$$S_a = \frac{1}{N_x N_y} \sum_{i=1}^{N_x} \sum_{j=1}^{N_y} \left[z\left(x_i,\ y_j\right)\right]$$

where N_x, N_y are the numbers of points in X- or Y-direction, and z (x_i, y_j) is the height difference between each point and the reference surface.

To determine surface roughness, produce and Sharklet samples were imaged with Zeiss 780 confocal microscope using the same procedure as in colloid imaging above. However, the surfaces used for roughness measurements did not have any retained colloids so only the reflected channel (633 nm laser line) was used. To determine roughness, minimum of 6 images per sample type were obtained, and roughness was analyzed and calculated using the topography function of ZEN 2010 D software (Carl Zeiss, Inc., Jena, Germany). To ensure the same optical conditions, all samples were imaged on the same day.

Selected samples (tomato, orange, apple, and lettuce abaxial) were also imaged with a 3D laser scanning microscope (KEYENCE, Osaka, Japan) to confirm the roughness values obtained with Zeiss 780 confocal microscope. Minimum of 3 images per sample type were obtained.

Both Zeiss confocal and KEYENCE microscopes converted the light intensity data of the image to corresponding height values. All images were 1st fitted to a plane to remove surface tilt. All data were filtered with a high-pass Gaussian filter with long wavelength cutoff λ_c at 80 μm to remove large-scale topography, which is less relevant to retention of 1-μm colloids, but affects the S_a value, and therefore may be misleading in search for correlation between roughness and colloid retention (for example, Berglund and others 2010). The choice of λ_c value is application-specific (Stout and others 1993) and in this study was based on the analysis of produce surfaces and dimensions of their microscopic features, which were smaller than the selected cutoff value. High-pass filter affects only low-frequency features above 80 μm, and therefore does not affect roughness features.

Surface hydrophobicity

Surface hydrophobicity was characterized via contact angle measurements and water retention experiments. The samples were cut into 2 × 2 cm squares with the surfaces as flat as possible.

Equilibrium contact angle was determined via sessile drop method: 3-μL droplets (5-μL droplets for spinach) of DI water were placed on the surface of interest and photographed with a high-resolution camera (Canon EOS T2i camera with Canon EF-S 18 to 55 mm f/3.5 to 5.6 IS II Lens and Raynox MSN-202 Macro Lens). Samples were prepared from different locations of the leaf or fruit, and 3 to 5 images per sample type were obtained. Images were analyzed using DropSnake plugin (Stalder and others 2006) for ImageJ software (Rasband 2015).

Water retention experiments were performed by immersing samples (n = 6) into 0.3 mM NaCl solution followed with fast sample withdrawal to reproduce the procedure done in batch experiments. Amount of retained solution and its distribution on

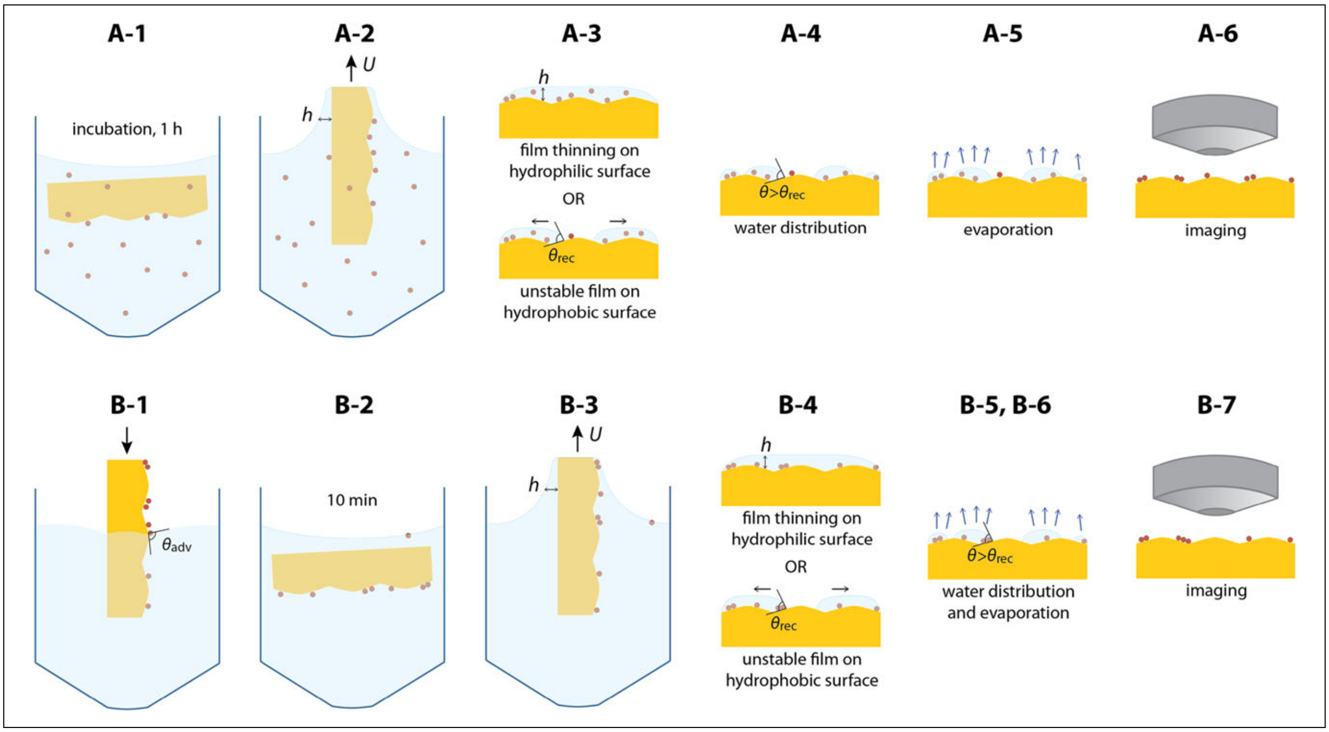

Figure 1–Schematic of processes taking place on produce surfaces during batch experiment (schematic A) and rinsing (schematic B).

sample surfaces were captured on photographs, which were used for measuring water retention area with ImageJ software. To enhance photo quality and method sensitivity, tomato, orange, lettuce, and spinach samples ($n = 3$) were immersed into 10 ppm colloid suspension, and water retention area was measured via fluorescence detection under UV lamp and Volocity software analysis. Additional details on this method are provided in Supplementary Information S1.

Contact angle characterizes hydrophobicity as a physicochemical property of the surface and reflects both the chemical composition of the surface and its roughness. Water retention measurements cover larger sample areas and characterize combined effects of hydrophobicity and topography.

Pearson correlation analysis

Pearson correlation analysis was performed using Origin 9.0 (OriginLab Corporation, Northampton, Mass., U.S.A.) for quantifying the correlations among the number of colloids retained per surface area, percentage of colloids retained as aggregates, contact angle, surface roughness, and water retention area.

Results

Colloid retention and removal from produce surfaces

Microscopy images of surface topography and colloid retention for the samples that were not rinsed are shown in Figure 2. Tomato, lettuce, spinach, and cantaloupe valley demonstrated distinct orderly microscopic surface features determined by the shape of cells of the epidermic layer, and Sharklet had a periodic artificial pattern. Orange, apple, and cantaloupe top showed more random shapes and distribution of microscopic surface features.

Colloid retention pattern on spinach adaxial (Figure 2) was found to be closely related to the surface features where colloids were retained preferably between the epidermal cells rather than on top of cell surfaces; spinach abaxial and lettuce adaxial also demonstrated similar colloid retention pattern. These findings are consistent with observations by others, for example, Warner and others (2008) found retention of *Salmonella* on spinach leaf mostly in the intercellular margins or in and around stomata. Predominant colloid and bacterial retention between epidermal cells has been also reported for other species such as Tyee savoyed-leaf spinach (Mitra and others 2009) and taro leaves (Ma and others 2011). In contrast, colloids retained only on the top of Sharklet features (Supplementary Information S2).

Quantitative colloid retention on produce and Sharklet surfaces is summarized in Figure 3: the numbers of colloids retained per image area (approximately 0.18 mm^2) were plotted for "not rinsed" (blue bars) and "rinsed" (red bars) samples. There are several trends in Figure 3. For the samples that were not rinsed, the highest colloid retention was found for apple and lettuce abaxial followed by spinach abaxial and lettuce adaxial. The least colloid retention was found for Sharklet. Rinsing efficiency also varied: higher percent of colloids were rinsed off the surfaces of tomato, orange, and cantaloupe compared to other surfaces.

For spinach abaxial and Sharklet, the average number of colloids after rinsing was slightly higher than before rinsing, which could be explained with the nonuniform distribution of colloids. Nonuniform colloid distribution was further enhanced during rinsing due to redistribution of colloids with water and moving contact lines and contributed to the slightly higher average numbers of colloids in rinsed samples.

We also analyzed and quantified the presence of colloid aggregates in the images (Figure 4), which often form as a result of contact line movement or evaporation. The aggregates were defined here as 2 or more colloids attached together. Percentage of colloid aggregates was determined as the ratio of the number of colloids present in aggregates to the total number of colloids in the image. Re-distribution of colloids during rinsing is supported by the aggregate analysis. Figure 4 shows that the percentage of retained colloids present as aggregates increased after rinsing, which confirms that colloids were moved and rearranged.

Produce surface properties

Surface roughness. Surface roughness values (S_a) obtained with 780 confocal microscope and KEYENCE microscope are shown in Figure 5A and Supplementary Information S3, respectively. The general trend is similar for both 780 confocal and KEYENCE microscopes. The error bars (that is, standard deviation) are large especially for the surfaces with greater roughness, likely due to the extensive heterogeneity of the produce surfaces. Large standard deviation could also be partially caused by the noise or artifacts from laser reflection, especially for the surfaces with high reflectivity such as lettuce and spinach. All observed surfaces can be divided into 2 relative roughness scales: small (tomato, orange, apple, Sharklet, with $S_a < 2\ \mu m$) and large (lettuce, spinach, cantaloupe, with $S_a > 3\ \mu m$).

Surface hydrophobicity. Equilibrium contact angle values are provided in Figure 5B, which shows that the samples are hydrophobic except for lettuce. Measurements on lettuce surface were difficult to conduct due to spreading of droplets. Droplets also spread with time on the surface of cantaloupe (faster compared to other hydrophobic samples) despite its high contact angle. It is likely due to the crevices on its surface where air gets trapped and displaced. Sharklet had the highest contact angle among all investigated surfaces. Overall, equilibrium contact angle measurement is not completely adequate to characterize produce hydrophobicity due to surface curvature, surface heterogeneity, droplet spreading, and so on, which reduce measurement accuracy.

Water retention on sample surfaces helps characterize hydrophobicity further because hydrophilic surfaces retain more water on their surfaces while water rolls off a hydrophobic surface (Wang and others 2014). Furthermore, water retention amount and pattern are more characteristic of receding contact angle and receding contact line movement along the surface (Blake and Ruschak 1997). Samples demonstrated distinct differences in water movement, retention, and distribution upon withdrawal from the solution. Measured water retention area is presented in Figure 5C as the percentage of sample surface occupied by water and illustrated in Figure 6. As discussed in Supplementary Information S1, the water retention area values may be overestimated for orange and underestimated for spinach adaxial.

Thickness of retained water differed between the samples and therefore could be visually compared. Water was present as thin water films on the surfaces of cantaloupe and spinach adaxial, although it was retained as discrete droplets on other samples. Orange, lettuce (adaxial and abaxial), and spinach abaxial had relatively tall droplets. Sharklet, tomato, and apple had similar water retention patterns (as few small droplets) and retained water amounts.

Clear association of water retention with topographic depressions was found for orange, lettuce (adaxial and abaxial), and cantaloupe. For spinach adaxial, larger water amount was retained around the veins of the leaf whereas water was mostly bounded by the veins in spinach abaxial.

Statistical correlations between colloid retention and surface properties. To quantify the correlations among the number of colloids retained (per surface area), percentage of aggregates, contact angle, surface roughness, and water retention area, we performed Pearson correlation analysis, with the results listed in Table 1. The apple scenario was excluded in Table 1 simply because colloid retention characteristics on apple surface were clearly different from all other surfaces, and the analysis results including apple set were shown in Table S1 (Supplementary Information S4). A significant positive correlation was found between the number of retained colloids and the percentage of aggregates, with the *r*-value (Pearson correlation coefficient) of 0.925 and *P*-value (significance) of 0.0003. In addition, the number of retained colloids was found to be negatively correlated with the contact angle of the surface with estimated *r*-value of −0.734 and *P*-value of 0.024, and positively correlated with the surface roughness (*r*-value of 0.648 and *P*-value of 0.059). Only a weak (and insignificant) positive correlation was found between the number of retained colloids and water retention area (*r*-value of 0.363 and *P*-value of 0.338). Similarly, the percentage of aggregates was found to be negatively correlated with the contact angle of the surface (*r*-value of −0.839 and *P*-value of 0.005) and positively correlated with the surface roughness (*r*-value of 0.668 and *P*-value of 0.049). There is weak and insignificant positive correlation between the

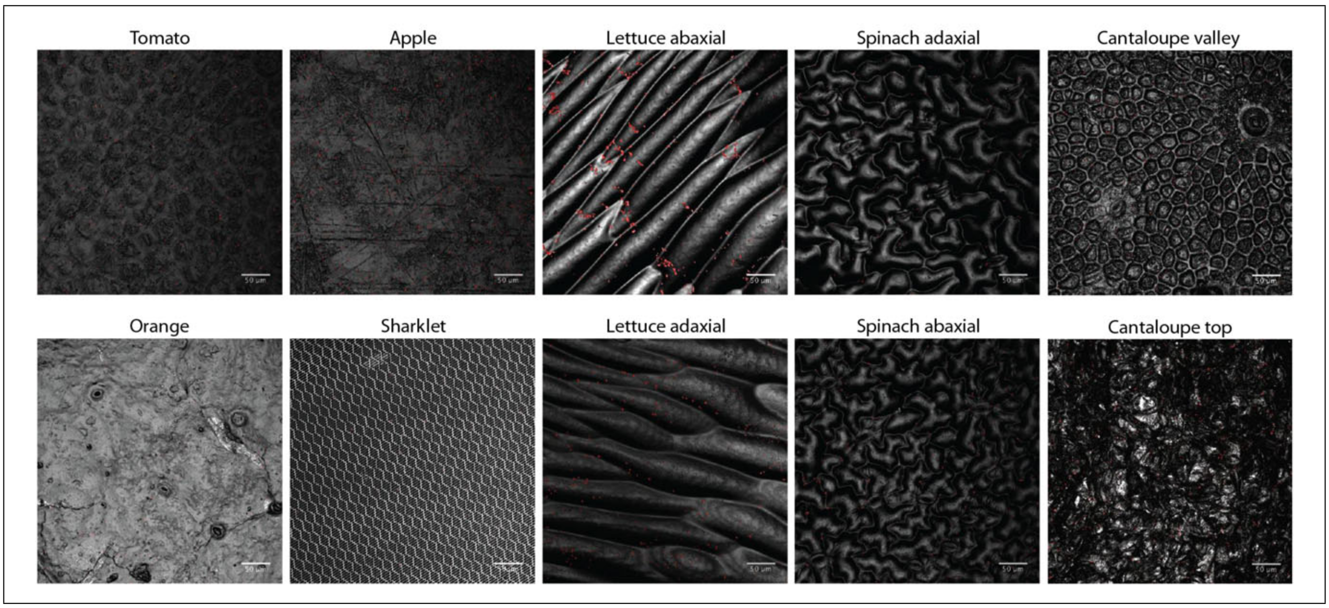

Figure 2–Images of colloid retention and topography. Scale bar is 50 μm.

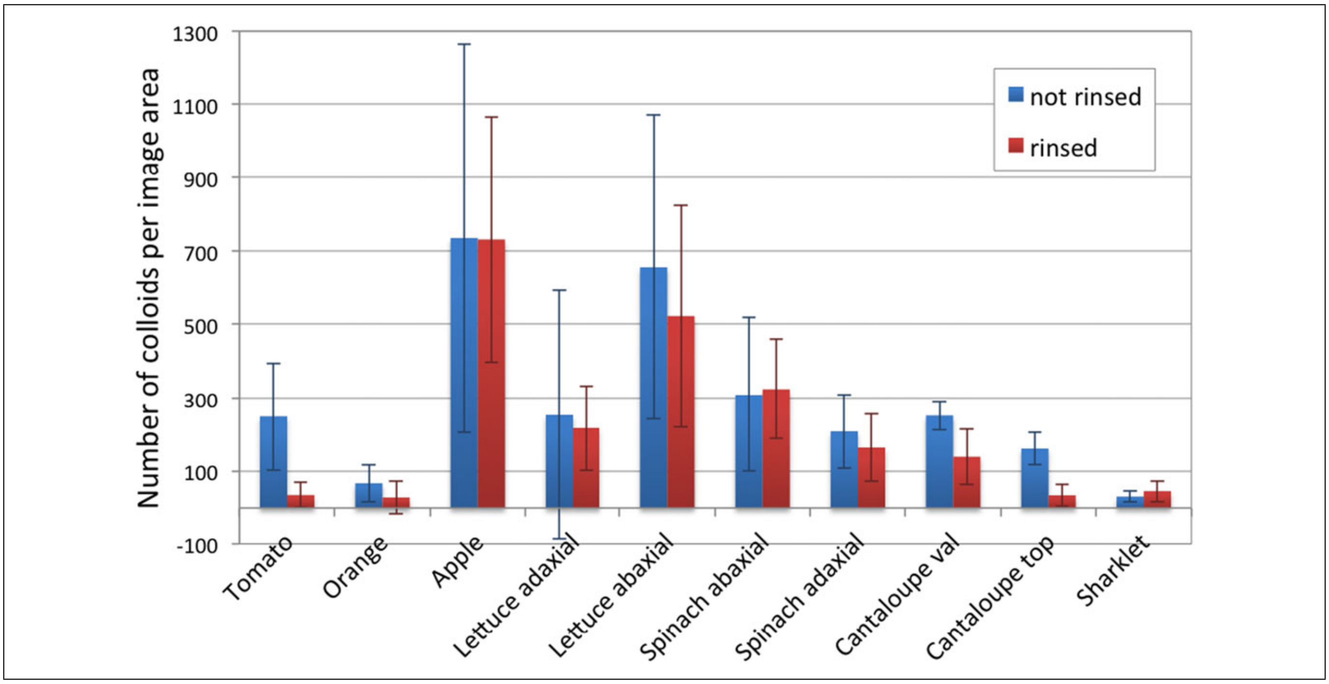

Figure 3–Numbers of colloids attached to produce surfaces per image area. Image area is equal to approximately 0.18 mm^2. The number of analyzed image areas per produce type ranged from 8 to 34. Error bars represent standard deviations.

percentage of aggregates and water retention area (r-value of 0.355 and P-value of 0.349). No significant correlations were found among the contact angle, surface roughness, and water retention area, except for surface roughness and water retention area with r-value of 0.730 and P-value of 0.025.

Discussion

Mechanisms leading to colloid retention and removal

Schematic A of Figure 1 illustrates the processes and associated mechanisms that affected colloid retention and spatial distribution in the samples that were not rinsed.

Initial colloid attachment to produce surface occurs in the suspension, that is, during incubation (Schematic A-1). This step is affected by the physicochemical properties of solution and colloid and produce surfaces (for example, ionic strength, surface charge, Hamaker constant and so on), which determine electrostatic and van der Waals interactions (for example, Hermansson 1999).

Upon sample withdrawal from the suspension (schematic A-2), a water film is formed. Film thickness depends on the withdrawal velocity and can be estimated by the LLD law for a plate as $h = 0.94aCa^{2/3}$, where $a = (\sigma/\rho g)^{1/2}$ is capillary length, $Ca = \eta U/\sigma$ is capillary number, g is gravitational acceleration, σ, ρ, and η are surface tension, density, and viscosity of liquid phase, respectively (for example, Quéré 1999). This expression is valid for $Ca < 10^{-2}$ (Seiwert and others 2011). In our experiments, capillary number and film thickness (Schematic A-2) are estimated as 1.2×10^{-3} and 29.3 μm, respectively. Film of such thickness does not affect attached colloids during withdrawal. However, these estimations are only valid for smooth surfaces. Films thicker than predicted by the LLD law have been reported for rough surfaces (Chen 1986; Krechetnikov and Homsy 2005; Seiwert and others 2011) due to modified boundary conditions (Krechetnikov and Homsy 2005) and liquid trapping between roughness features (Seiwert and others 2011). Krechetnikov and Homsy (2005) also reported increased film stability on surfaces with higher roughness. Concurrent film formation and drainage during sample withdrawal (Schematic A-2) and the lifetime of the film (before it ruptures) depend on surface and solution properties (Krechetnikov and Homsy 2005) and result in different water amounts on produce surfaces by the end of step A-2.

As the film drains (Schematic A-3), its further behavior also depends on surface and solution properties. On a hydrophobic surface, the film is unstable: it ruptures and dewets the surface. Formed triple-phase contact line will recede across the surface and exert capillary forces on attached colloids. Contact angles of colloids and surfaces are central parameters defining capillary force (for example, Aramrak and others 2011; Lazouskaya and others 2013). Capillary forces can hold colloids on the contact line and move them along the surface leading to their redistribution and causing aggregation (Kralchevsky and Denkov 2001). On a hydrophilic surface, the film persists on the surface longer: it thins under gravity, eventually becomes unstable, and then breaks into droplets. In case of thin films, colloids already attached to the surface and embedded in the film will be little affected by film thinning (Schematic A-3). Also, contact lines with small receding contact angles are not as efficient in moving colloids (Lazouskaya and others 2013) and affecting colloid distribution. Therefore, receding contact line behavior and receding contact angle value are key factors controlling colloid movement along the sample surface at this step.

Water retention and distribution (Schematic A-4) depend on receding contact angle, topography, and roughness of the surface. Water (contact line) starts receding along the surface when dynamic contact angle reaches receding contact angle value (Blake and Ruschak 1997). Therefore, more water retains on a surface with a lower receding contact angle. Topographic depressions become locations for water trapping due to gravity. Also,

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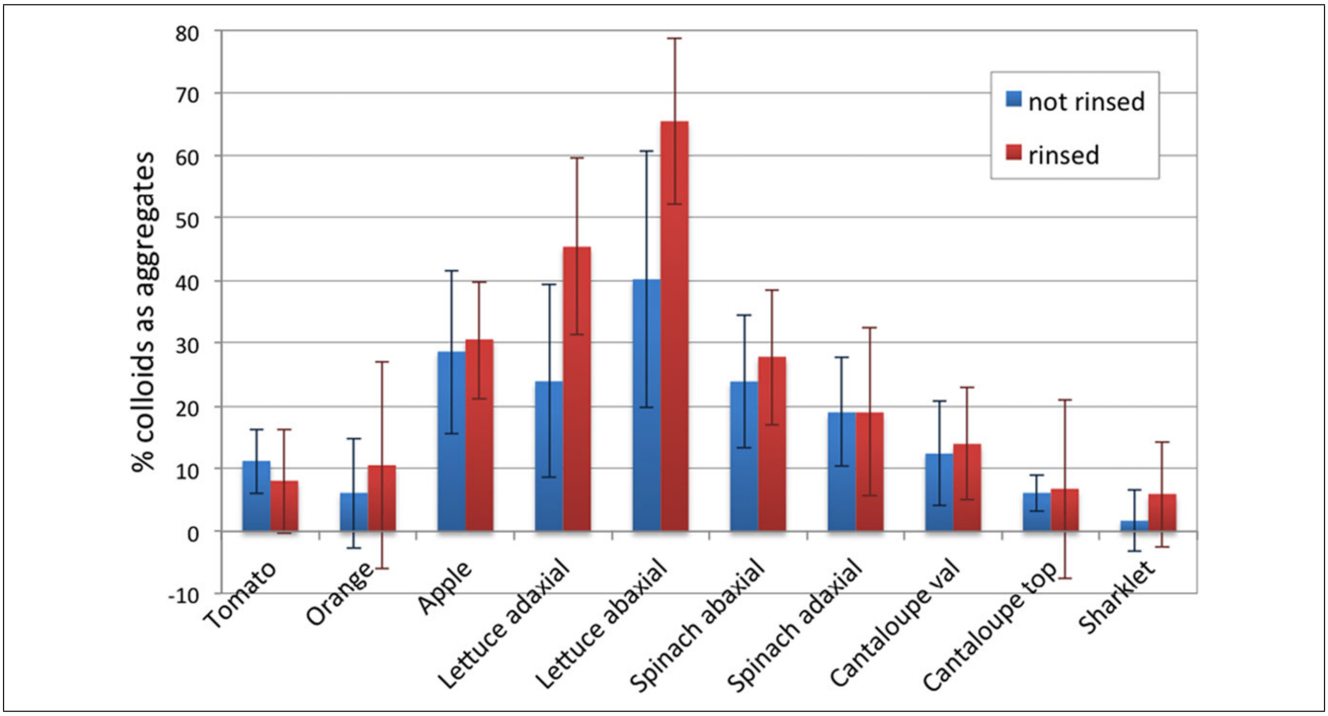

Figure 4–Percentage of colloids present in the image as aggregates, that is, 2 or more colloids aggregated together. The number of analyzed image areas per produce type ranged from 8 to 34. Error bars represent standard deviations.

topography can affect local dynamic contact angle (so that it does not reach the receding contact angle value) thus enhancing water retention in respect to topographical features (Hejazi and Nosonovsky 2013). Contact line can be pinned on roughness features, which lowers receding contact angle (for example, Bhushan and Jung 2008). Therefore, roughness effect on receding contact angle and film stability results in higher water retention. In our work, retention of residual colloid suspension is an additional source of colloids on the surface; therefore, analysis of water retention (including water retention area and droplet size and thickness) can help estimate addition of colloids during drainage to the ones retained on surfaces during incubation (A-1).

Evaporation is another process that affects colloid retention pattern on produce surfaces. Droplet evaporation from produce surfaces (Schematic A-5) starts with movement of dispersed colloids to the contact line due to evaporative flux in the droplet. Colloids arrive at the contact line, form aggregates, and pin it. When the contact line recedes, colloids either move with contact line or

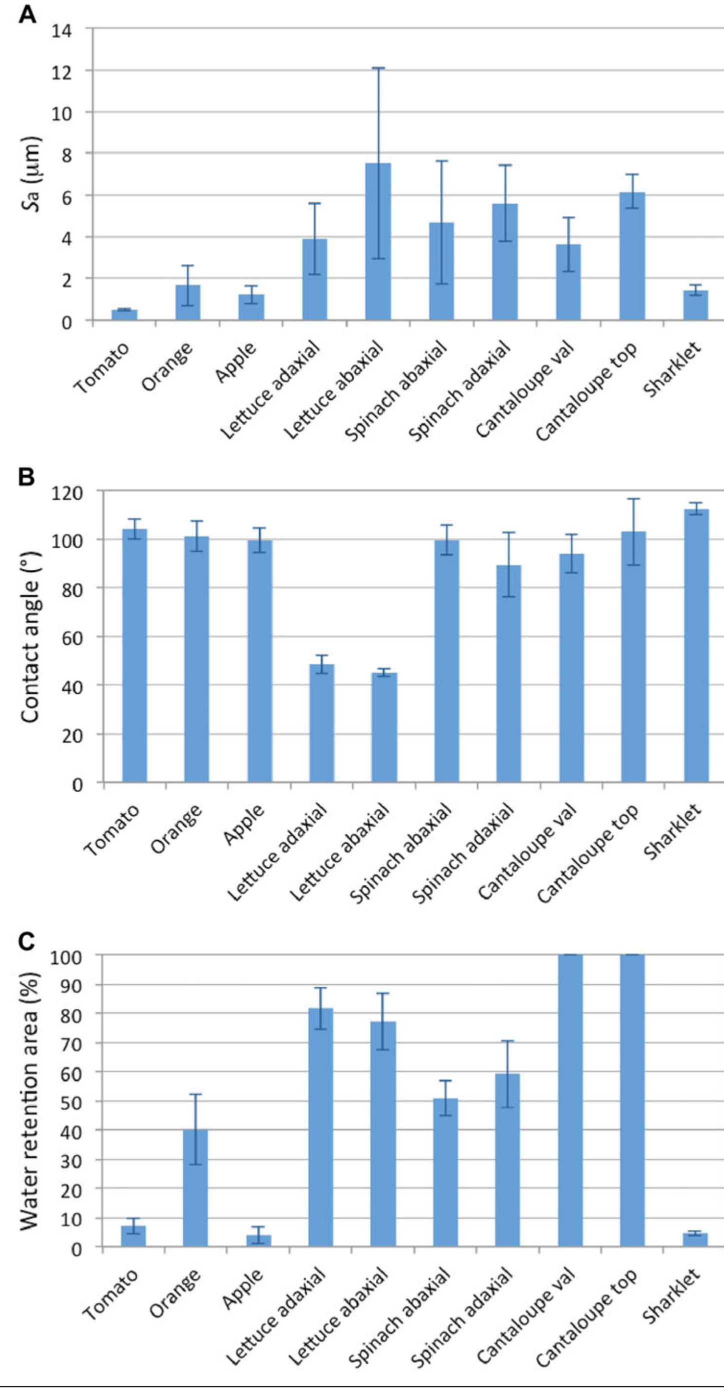

Figure 5–Produce and Sharklet surface properties: (A) surface roughness S_a obtained with 780 confocal microscope (data were filtered with a high-pass Gaussian filter at 80 μm); (B) contact angle; (C) percentage of sample area covered with water upon sample withdrawal. Error bars represent standard deviations.

retain on the surface (for example, Eral and others 2013). Pinning of the contact line is most effective when surfaces have defects, that is, pinning sites such as roughness features. Although aggregates can also form during step A-1, we mostly attribute aggregate formation to contact line movement (A-3) and evaporation (A-5) due to capillary bridge forces between the particles on the contact line (for example, Kralchevsky and Denkov 2001).

Additional events occur during rinsing, as shown in Schematic B of Figure 1. Rinsing can cause some colloids to detach with advancing contact line when the sample is immersed in the colloid-free solution (Schematic B-1). Rinsing also reproduces all the processes that occur to the samples prior to rinsing (schematics B-3, B-4, B-5, and B-6), but water phase used during rinsing is colloid-free. Sample withdrawal (B-3), water movement (B-4), retention (B-5), and evaporation (B-6) create additional movements of contact line along the sample surface and cause re-distribution of colloids.

Parameters affecting colloid retention on produce surfaces

Figure 3, 5A-C, and 6 show that the impact of different parameters (hydrophobicity, roughness, and topography) on colloid retention differs depending on sample type. However, general trends can be observed if apple is excluded from the analysis (Table 1). We found that colloid retention is positively correlated to surface roughness and negatively correlated to contact angle. Roughness is particularly critical to colloid retention on some samples (for example, low retention is associated with low roughness for tomato, orange, and Sharklet, and high retention—with high roughness of lettuce abaxial), but other factors in addition to roughness are also found to be important to colloid retention. These results are consistent with the study by Wang and others (2009), in which they conducted experiments with orange, apple, cantaloupe, and avocado and found more bacterial retention for produce with higher surface roughness. Wang and others (2007) have also acknowledged that other factors in addition to surface roughness affect bacterial reduction rate on produce surfaces.

Although water retention area is only weakly correlated to colloid retention (Table 1), qualitative agreement is observed for the amount (thickness) of retained water and colloid retention (Figure 3). In Figure 6, we distinguish 3 groups of samples according to their film thickness: large film thickness (orange, lettuce, and spinach abaxial), small film thickness (spinach adaxial, cantaloupe), and samples with very low water retention, for which film thickness is not relevant (tomato, apple, and Sharklet). Samples with large nonuniform film thickness such as spinach abaxial and lettuce have high colloid retention characterized with large standard deviation. Spinach adaxial and cantaloupe are the cases where water covers large areas as a thin film, which results in uniform distribution of colloids characterized by low colloid retention and low percentage of aggregates. Orange has lower colloid retention because colloid retention was measured in the sample's center whereas water mostly retained at the sample's edges.

All 3 factors, surface roughness, contact angle, and topography, affect water retention and film thickness. According to Krechetnikov and Homsy (2005), surface roughness promotes film stability, which leads to slower drainage and higher water retention area. Indeed, samples with relatively high roughness (lettuce, spinach, and cantaloupe) have higher water retention area compared to

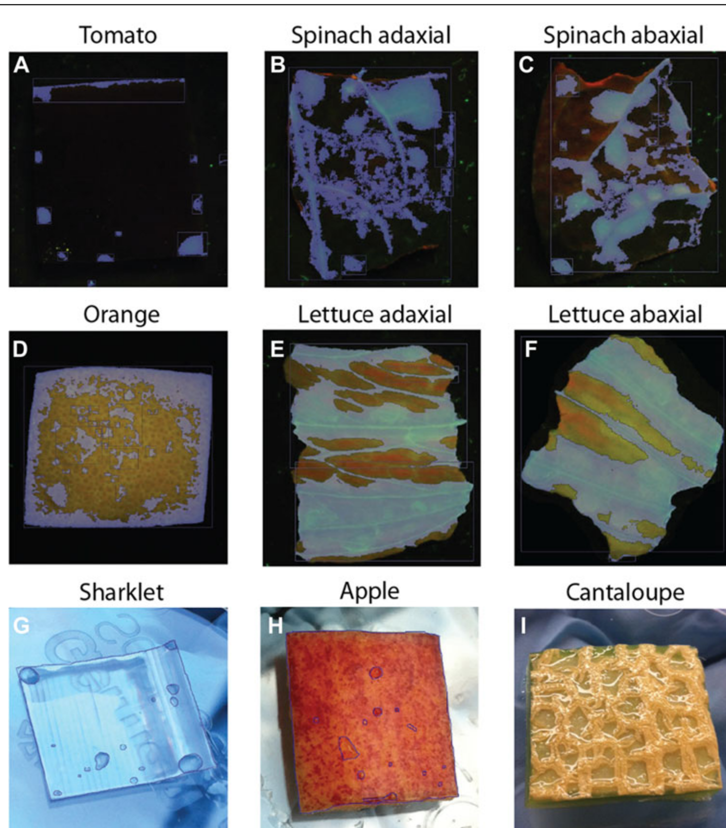

Figure 6–Highlighted water retention area measured with fluorescence detection method and Volocity software (A-F) and with ImageJ software (G, H). Water film is shown on cantaloupe sample (I).

Table 1–Pearson correlation analysis between colloid retention and surface properties (excluding results for apple samples).

		Colloids per area	Aggregates (%)	Contact angle (°)	Surface roughness (S_a, μm)	Water retention area (%)
Colloids per area	*r*-value	1	0.925	−0.734	0.648	0.363
	P-value	–	0.0003	0.024	0.059	0.338
Aggregates (%)	*r*-value	0.925	1	−0.839	0.668	0.355
	P-value	0.0003	–	0.005	0.049	0.349
Contact angle (°)	*r*-value	−0.734	−0.839	1	−0.551	−0.469
	P-value	0.024	0.005	–	0.124	0.203
Surface roughness (S_a, μm)	*r*-value	0.648	0.668	−0.551	1	0.730
	P-value	0.059	0.049	0.124	–	0.025
Water retention area (%)	*r*-value	0.363	0.355	−0.469	0.730	1
	P-value	0.338	0.349	0.203	0.025	–

the samples with relatively low roughness (tomato, orange, apple, and Sharklet). Although a thinner film is expected for hydrophilic lettuce compared to the rest of hydrophobic samples (for example, Bacri and Brochard-Wyart 2001), it is not observed, likely due to topography effect. Samples without considerable large-scale topography (tomato, apple, and Sharklet) do not support additional water retention in contrast to the samples with distinct topographic features such as lettuce. Tomato, apple, and Sharklet have low roughness, high contact angle, and smooth topography, which all promote unstable film and therefore less water retention. Orange has similar roughness and contact angle, but complex topography and therefore has thicker film retained, mostly associated with topographic depressions. Lettuce, spinach, and cantaloupe have higher roughness (and therefore more stable film) as well as complex topography. Nevertheless, hydrophilic lettuce has larger droplets, and hydrophobic spinach adaxial and cantaloupe have thin water films. The reason for such water behavior is not clear, but it could be due to water spreading and imbibition within the veins of spinach or cantaloupe crevices.

Colloid retention on apple surfaces does not follow the proposed mechanisms. Apple samples demonstrate high colloid retention at low water retention despite its high contact angle, low roughness, and smooth topography. Observed apple's high colloid retention and low rinsing efficiency can be attributed to physicochemical (electrostatic, van der Waals, and hydrophobic) interactions between apple surfaces and colloids. Apple surfaces were covered with wax, and therefore colloid retention could be due to physicochemical interactions between colloids and the wax. Because of common use of wax in produce, such interactions would be of additional interest in the future. Overall, we did not consider physicochemical interactions of colloids and produce in this work due to lack of certain produce parameters (such as surface charge and Hamaker constant), but studying these interactions should remain a priority in the field of produce safety.

Rinsing efficiency

Rinsing efficiency differs between produce samples. Colloid retention (Figure 1, Schematic A) is composed of attached colloids (A-1) and colloids retained with residual water/suspension (A-4). Colloid retention on rinsed samples (Figure 1, Schematic B) includes attached colloids (A-1) without contribution from residual suspension (because samples are not dried prior to rinsing) minus colloids removed with the advancing contact line (B-1). Therefore, the difference in retained colloids before and after rinsing is due to removing residual suspension and advancing contact line action. The contributions of these 2 processes are related: for surfaces with high water retention advancing contact line acts only on the water-free areas and thus is less important while for surfaces with low water retention advancing contact line moves across larger area. Therefore, for tomato, orange, apple, and Sharklet (surfaces with small residual water area), colloid rinsing is largely influenced by the advancing contact line. For lettuce and spinach, colloid rinsing is mostly due to residual water removal, but heterogeneous distribution of water (Figure 6) results in nonuniform colloid distribution and large error bars and makes this conclusion in Figure 3 less clear. For cantaloupe, most colloid removal during rinsing was due to residual water removal although other processes (such as air trapping and water retention in Cassie-Baxter state, Supplementary Information S2) can also be important. Overall, residual water retention and behavior are important in evaluating rinsing efficiency and require additional investigation.

Conclusion

In this work, we investigated colloid retention and removal on a broad range of fresh produce surfaces and an artificial surface Sharklet. We characterized a set of physicochemical properties of produce surfaces including surface roughness and hydrophobicity (that is, contact angle and water retention area) and investigated their roles in colloid retention. Contrary to previous reports, surface roughness was not the key parameter in colloid retention on all produce surfaces. Instead, our results clearly indicate that it is unlikely that any single parameter can solely control colloid retention on fresh produce. Rather, we found that water retention amount, which depends on a combination of produce properties (roughness, topography, hydrophobicity), was the strongest indicator of colloid retention for all samples except apple. Our results suggest that understanding water behavior and minimizing water retention on produce will reduce produce contamination. Rinsing was found ineffective for colloid removal for a number of produce samples (apple, lettuce, and spinach). The reasons for such inefficiency are complex due to interactions of multiple parameters and processes involved and are a subject of future work. Although water retention reproduces general colloid retention trend, surface properties, and physicochemical interactions have to be further investigated to improve prediction of colloid retention and removal and to devise effective strategies for produce contamination prevention or effective cleaning treatments.

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Authors' Contributions

V. Lazouskaya, G. Wang, and Y. Jin designed the study and wrote the manuscript. V. Lazouskaya and T. Sun performed experimental work. L. Liu and G. Wang performed calculations and statistical analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

The file containing Supplementary Information S1-S4 is available online.

Figure S1. Illustration of water retention measurements using fluorescence detection method including: (A) sample photograph taken after sample withdrawal from colloid suspension, (B) sample photograph taken under UV lamp, and (C) Volocity image with identified water retention areas.

Figure S2. Colloids on the tops of Sharklet features.

Figure S3. Roughness S_a of selected produce obtained with KEYENCE microscope.

Table S1. Pearson correlation analysis between colloid retention and surface properties (including values for apple samples).