Food Surface Texture Measurement Using Reflective Confocal Laser Scanning Microscopy

S. SHEEN, G. BAO, AND P. COOKE

ABSTRACT: Confocal laser scanning microscopy (CLSM) was used in the reflection mode to characterize the surface texture (roughness) of sliced food surfaces. Sandpapers with grit size between 150 and 600 were used as height references to standardize the CLSM hardware settings. Sandpaper particle sizes were verified by scanning electron microscopy (SEM). The mean amplitude (in micrometers) of surface variation along line segments of the scanned sandpaper topographical image sets showed very close agreement between the measured result and the grit particle size (based on the U.S. Coated Abrasive Manufactures Inst. [CAMI], standard). The verified instrument settings were then used to measure the surface texture of mechanically sliced food surfaces, including cooked ham, salami, and cheese. Sliced food surface texture parameters of Ra (average height of a line segment), Rs (surface area ratio), Pa (average height on a region of interest), and Pq (root-mean-square height on a region of interest) were evaluated by this method. Values of the surface roughness of sliced ham, salami, and cheese were found to be comparable to the range of dimensions of selected sandpapers. The CLSM method may be useful for other surface texture measurements and to investigate the impact of food surface texture on microbial adhesion or attachment, which might play a significant role in microbial transfer from one surface to another.

Keywords: confocal laser scanning microscopy, food surface texture, roughness

Quantitative surface measurement is an important field of engineering and applied sciences, including food technology. Due to the complexities of texture and surface topography, no universal method of measurement and/or instruments, which could be applied to all kinds of surfaces, are available for accurate surface characterizations such as measured metal surfaces and wear of electrically powered cutting blades. Recently, food texture studies, including surfaces and internal matrices, are gaining more attention (Hershko and others 1998; Bouchon and others 2003; Morton and others 2003; Nicolas and Paques 2003; Quevedo and Aguilera 2004; Butz and others 2005; Chen 2007; Yang and others 2007) in an effort to understand and improve food processing and quality. For food safety, the characteristics of a food surface may play an important role in harboring pathogens. The adhesion or entrapment of bacteria within or on foods and/or machine parts or other contact surfaces associated with foods (Taylor and others 1998; Verran and others 2000) may also impact the transfer of microbes during processing, especially slicing. The selection of a reliable method for surface texture quantification could depend on various factors such as image size and resolution, scanning speed, 2-D/3-D image, sample geometry, surface texture preservation, and other factors (Yang and others 2007).

To investigate the surface of a target material, it must be accurately “scanned” and characterized in terms of measurable parameters (for example, roughness). Using confocal microscopy, Lange and others (1993) analyzed surface roughness (fracture) to provide a convenient means of acquiring 3-D descriptions of objects (cement paste, mortars). Brown and others (1994) patented a “patchwork” method (3-D triangular patches) to simulate the specimen surface as a function of patch size or scale of observation or interaction and claimed that their method may provide the most accurate surface area calculation (a numerical approximation). The microscope “optically” sections the surface and a computer transforms a series of sections into digital images and a topographic map. A roughness parameter, Rs (that is, actual surface area/projected surface area), was computed to describe surface roughness. Wendt and others (2002) developed computer software to analyze the brittle fracture surfaces and wire-eroded surfaces of low-alloyed steel and stainless steel. The surface data were obtained from confocal laser scanning microscopy (CLSM). They found that global topometry values such as the normalized surface area, the mean linear profile segment length, and the fractal dimension were significantly dependent on the imaging conditions and computer algorithm. Pedreschi and others (2002) demonstrated that a scanning laser microscope (SLM) can be used to measure the surfaces of different foods and how parameters describing the surface morphology can be obtained from length- and area-scale fractal analyses. The SLM measured heights at discrete points as a function of position in a regular grid with a finest spatial resolution of 25 μm. Pedreschi and others (2002) also used a SLM to study the surfaces of 3 kinds of commercial chocolates. Data of measured surfaces were analyzed by scale-sensitive fractal analysis (SSFA) using linear and area tiling and by conventional statistical analyses of roughness. Using this approach, they were able to characterize the surface roughness and changes in topography due to bloom caused by the recrystallization of cocoa butter fat under noncontrolled temperature conditions. Briones and others (2006) also applied the SSFA to describe the surface roughness of bloomed chocolate. Wessel and others (2003) demonstrated that the unevenness

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and steep edges of an object may negatively impact the images obtained by reflection CLSM. However, they concluded that reflection CLSM is an underestimated, quantitative, topographic imaging tool and a real alternative compared to scanning electron microscopy (SEM) and atomic force microscopy (AFM) for surface texture measurement applications. Bouchon and Pyle (2004) studied the texture of potato chip surfaces of 3 different formulations with CLSM and reported that root-mean-square roughness may be used as a discriminator. However, the higher order moments, skewness and kurtosis, showed no significant difference between the distinct topographies. Tomovich and Peng (2005) discussed the importance of quantitative surface measurement in engineering using CLSM, a nondestructive and versatile technique for 3-D surface features. They also pointed out that there is little documentation on standard CLSM hardware settings to capture adequate images for 3-D surface measurement, and they described a reliable 3-D image analysis system for image processing and surface measurement, regardless of surface reflectivity or surface finishes on engineering surfaces and small particles. Chen and others (2006) used CLSM to examine the surface topography of heat-set whey protein gels and described the surface texture in terms of root-mean-square surface roughness and arithmetic surface roughness. Flores and others (2006) used CLSM to characterize the roughness of HDPE cutting boards and to study the transfer of pathogens (Escherichia coli O157:H7) between surfaces. It was equipped with a carbon steel hollow ground blade of 12-inch (305-mm) dia that rotates at 300 rpm (one fixed speed). The blade was used about 6 mo for other meat slicing studies and prior to the present study thoroughly cleaned in detergent, Bac-Down Detergent Disinfection (Decon Labs Inc., Bryn Mawr, Pa., U.S.A.), at room temperature for 1 h, then autoclaved for 30 min. The clean blade was remounted into the unit and 2 to 3 slices of ham (68% moisture, 2% fat), 2 slices of salami (62% moisture, 15% fat), and 2 slices of cheese (American cheddar cheese, extra sharp), were prepared, each 2 to 3 mm thick. The contact time of blade per sliced sample was about 2 s or 5 revolutions per cut, which resulted in a relatively smooth sliced sample surface. Three types of sliced foods were examined using the CLSM, which are listed as follows:

(1) **Fresh-cut samples:** the sliced food samples were immediately scanned per the CLSM settings with the surfaces retaining at their originally cut texture. Six areas at different locations on the slice were tested.

(2) **Glutaraldehyde-treated (protein crosslinked) samples:** Two-centimeter square areas cut from slices of cheese, ham, and salami were immersed in 20 mL of 2.5% glutaraldehyde-0.1 M imidazole buffer solution (pH 7.2) and stored for 2 d in sealed vials before imaging. Three sets of images taken from different sample locations were tested.

(3) **Dried samples:** After preserving samples in glutaraldehyde for fixation, the 2-cm areas of slices were washed in imidazole buffer, and dehydrated by exchange with 20 mL of graded ethanol solutions, from 50%, 80%, to absolute ethanol (2 changes at each

### Materials and Methods

#### Confocal microscope

Surfaces of the sandpapers and food sample slices were examined and imaged using a model IRBE microscope integrated with a model TCS-SP confocal scanner head (Leica Microsystems, Mannheim, Germany). Samples were mounted in a universal stage, on slides or within microwell dishes (MatTek Inc., Ashland, Mass., U.S.A.), and then illuminated with the 488-nm line from an argon laser through an RSP500 dichroic filter. Reflection from sample surfaces was collected with a long working distance (1.15 mm) 20× lens (numerical aperture = 0.5) in a single, 8-bit channel and the optional detector pinhole; begin and end limits of the vertical series were set visually in the Z-wide scan control. Gain and black levels for the channel 2 photomultiplier were adjusted through digital control in the glow/over/under look-up-table and two 512 × 512 pixel frames were averaged in each optical section. Image stacks with the optional number of sections for maximal z-resolution (for the lens) were transformed into topographical images for measurements in the Leica LCS Materials software package or converted to 3-D images.

### Sandpapers

Four sandpaper grades, Norton Brand manufactured by Saint-Gobain Abrasives Inc. (Hamilton, Ontario, Canada), were evaluated as size standards for surface roughness measurement. Sandpapers included 2 very fine grit sizes and 2 super fine grit sizes, which provided a close match to the particle size range for surface roughness of the sliced food samples. The grit sizes and average particle diameters are shown in Table 1. The 400 mm² (20 × 20 mm) pieces of sandpaper were mounted on carbon adhesive tabs fixed to glass microscope slides and sputter coated with a thin reflective layer of gold before imaging by CLSM. Stacks of optical sections were collected from surface areas and converted to topographical images using LCS Software (Leica Microsystems). Numerical values of surfaces were computed using the LCS Materials software for surface analysis and roughness measurements.

The CLSM settings, discussed in the previously section, were critically adjusted to obtain the measured sand paper particle size for each CAMI number. The particle size is the average amplitude (in micrometers) of results from 24 line segments (4 line segments per sample × 6 samples).

### Sliced food surface preparation

A retail-scale mechanical slicer (Globe Model 3500, Globe Food Equipment Co., Dayton, Ohio, U.S.A.) was used to slice the food samples. It was equipped with a carbon steel hollow ground blade of 12-inch (305-mm) dia that rotates at 300 rpm (one fixed speed). The blade was used about 6 mo for other meat slicing studies and prior to the present study thoroughly cleaned in detergent, Bac-Down Detergent Disinfection (Decon Labs Inc., Bryn Mawr, Pa., U.S.A.), at room temperature for 1 h, then autoclaved for 30 min. The clean blade was remounted into the unit and 2 to 3 slices of ham (68% moisture, 2% fat), 2 slices of salami (62% moisture, 15% fat), and 2 slices of cheese (American cheddar cheese, extra sharp), were prepared, each 2 to 3 mm thick. The contact time of blade per sliced sample was about 2 s or 5 revolutions per cut, which resulted in a relatively smooth sliced sample surface. Three types of sliced foods were examined using the CLSM, which are listed as follows:

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### Table 1—Mean amplitude of sandpaper particles using roughness profile.

<table>
<thead>
<tr>
<th>Sandpaper (grit grade)</th>
<th>CAMI* standard average particle diameter (μm)</th>
<th>Measurement mean amplitude (μm)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 (very fine)</td>
<td>92.0</td>
<td>94.38 ± 6.28</td>
</tr>
<tr>
<td>220 (very fine)</td>
<td>68.0</td>
<td>63.07 ± 6.30</td>
</tr>
<tr>
<td>400 (extra fine)</td>
<td>23.0</td>
<td>35.24 ± 2.55</td>
</tr>
<tr>
<td>600 (extra fine)</td>
<td>16.0</td>
<td>19.00 ± 1.77</td>
</tr>
</tbody>
</table>

*aCAMI is the U.S. Coated Abrasive Manufacturers Inst., now a part of the Unified Abrasives Manufacturers’ Assn.

^Sample size = 4 line segments/sample × 6 samples from different sandpaper.
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concentration). Samples were critical point dried from liquid CO₂ in a critical point dryer (Model DCP-1, Denton Vacuum Inc., Cherry Hill, N.J., U.S.A.). The dried sample surfaces were scanned with CLSM. Five sets of images taken from different locations were collected. Although food processing typically does not involve (1) and (3) operations, these 2 sample treatments are typical of what would be required for surface measurements by traditional microscopic imaging methods.

Surface texture evaluation

The following 2 methods of image processing and data analyses techniques were used per ASME (1995) standards. The bundled LCS software functions were selected to perform this calculation.

(1) Measuring the height along a line segment: a topographical image is the prerequisite for surface texture evaluation. For height profile analysis, the length and position of the line segment can be randomly selected or changed to the specified positions of a topographical image if needed. Statistical values and graphs may be recorded during the computation of roughness profile for later use. The mean roughness, Ra, of a line segment is the arithmetic average of the absolute value of the profile ordinates within the measured section (with respect to the average height, \( \bar{Z} \)) and can be calculated by the following equation, according to DIN EN ISO 4287 (1998):

\[
Ra = \frac{1}{l} \int_0^l |Z(x)| \, dx \quad \text{with} \quad Z(x) = Z(x) - \bar{Z}.
\]  

(1)

In terms of a digital expression as used by Pohl and Stella (2002), the equation becomes:

\[
Ra = \frac{1}{N} \sum_{i=1}^{N} |Z_i|
\]  

(2)

Six surface topographical images of each sample, including sandpapers and sliced foods, were collected and 4 line segments of each topographical image were evaluated. Therefore, a total of 24 line segment data were used to obtain the average Ra value and standard deviation.

(2) Measuring roughness within a region of interest (ROI): a computer algorithm (LCS materials software package, Leica Microsystems, Exton, Pa., U.S.A.) was used to approximate the surface area of a topographical image.

\[
Rs = A^* / \bar{A} = \frac{1}{\bar{A}} \int_0^A dA \cdot A
\]  

(3)

For the average height roughness (Pa) of a ROI:

\[
Pa = \frac{1}{A} \int_0^A |Z_{x,y}| \, dA \quad \text{with} \quad Z_{x,y} = Z(x,y) - \bar{Z}
\]  

(4)

For the root-mean-square roughness (Pq) of a ROI:

\[
(Pq)^2 = \frac{1}{A} \int_0^A Z_{x,y}^2 \, dA \quad \text{with} \quad Z_{x,y} = Z(x,y) - \bar{Z}
\]  

(5)

where

\begin{align*}
\bar{A} & : \text{the projected surface area (Euclidean surface)}; \\
\bar{l} & : \text{the straight length of line segment}; \\
N & : \text{the total pixel number over a line profile}; \\
Z_{x,y} & : \text{the height corresponding to a point with respect to mean height value of a line profile and ROI, respectively}.
\end{align*}

Similarly, the digital expressions for Pa and Pq can be found from Pohl and Stella (2002).

Measurement of particle height (grit) of sandpaper by SEM

The grit size of the sandpapers was also measured by a completely independent method, using SEM. The height of particles in topographical images was calculated from trigonometric principles. By using the difference in slope distance, measured from the same 2 feature points on the axis of rotation, one near the peak of the particle and the other at the base, in pairs of images of tilted samples at 0° and 30°, the slope angle was obtained. The height of a particle was derived finally from the tangent of the slope angle. Using SEM to measure particle size involves several tedious steps, including searching for the individual particles (free standing with no subtending or superimposed particles) and taking images at 2 angles. Six samples were obtained by this method to verify particle size.

Roughness measurement reproducibility by CLSM

The repeatability of roughness data was examined by using 2 methods with the 220-grit sandpaper: (1) same area and the same begin and end positions on the z-axis; (2) same area and each begin and end limits reset, visually. The sandpaper was placed at a fix location for scanning, one sample for (1) and another sample for (2) measurements. In (1) scenario, the settings of instrument were unchanged and several measurements were taken for analyses at 5-min intervals. In (2) scenario, the settings were reset for each vertical stack of optical sections, which were collected at several different times within a 2-h period.

Results and Discussion

Surface texture may be expressed in 2 ways: qualitatively, as a graphic image in which surface features appear as gradients of visual contrast, or quantitatively, as a 3-D image with relative heights in an elevation map. The CLSM provides dimensions for both 2- and 3-D images of surfaces. A very useful parameter in the surface roughness measurement using CLSM is the mean amplitude of the topography. The CLSM instrument settings were set to resolve the dimensions of the particles, and the values were then applied to other surface measurements. The measurement of the mean amplitudes of 4 sandpaper samples was consistent with the average particle diameter of the sandpapers per CAMI standards with a correlation of 0.982 (Table 1).

The surface texture of 4 sandpapers having grit grades of 150, 220, 400, and 600 showed different patterns in the 2-D images (Figure 1). It is obvious that the grit grade increases as the surface becomes finer (less coarse). The image stacks of each grit size were converted to topographical images for qualitative surface measurement. The computed line segment (Ra) and area roughness (Rs, Pa, and Pq) results of the 4 sandpaper specimens are presented in Table 2. The roughness values of each specimen are the average of 24 and 6 measurements for line segment and area (ROI), respectively. It was expected that the surface roughness results would increase when the grit size of the sandpaper decreased. However, the confocal results (Ra and Rs) did not show significant differences (\( P > 0.05 \)) in surface roughness between the sandpaper grit size 150 and 220. The Pa and Pq did have small increase from grit 220 to 150. Since the actual surface area of the specimen was estimated by the geometric construction of the surface, 2 close grit sizes of sandpaper might have similar surface roughness values because the particle distribution pattern obscures possible difference. The CLSM topography and the amplitude graphs (profiles) at the position along the drawn line, distinguished by color at 4 straight-line locations, of the sandpaper grit 150 is illustrated in Figure 2. Similar results were obtained for other grit sizes but those data are not shown. The mean
amplitude was averaged to the total pixels on each profile. The light and dark shades of the topography indicate surface peaks and valleys, respectively. The SEM images show particle size and distribution in sandpaper 150 (Figure 3), where the height of a single particle was calculated by stereo projection and the average height of the sandpaper particles calculated from 6 measurements. A challenge was to select stand-alone particles. Also, sample sizes scanned by SEM are relatively small and imaging may alter the surface integrity, especially in soft surfaces. For the selected particles, the calculated particle sizes in SEM images closely matched the CAMI grit size.

The repeatability of Ra and Rs measurement using the same sandpaper area (220 grit) and the same start and stop showed results of 5 repeated measurements to be 7.04 ± 0.126 μm (1.8% SD, based on mean) and 2.58 ± 0.097 (3.7% SD), respectively. The results of the same area and each start and stop limits reset are 7.34 ± 0.074 μm (1.0% SD) for Ra and 2.58 ± 0.037 (1.5% SD) for Rs. These data have demonstrated that the surface texture measurements by CLSM are statistically reproducible. Table 2 further showed the 2nd moment of sandpaper surface roughness in terms of Pa and Pq, which indicated that the bigger the particle size, the larger the roughness parameter of Pa and Pq. In general, Rs is the least sensitive (a narrow range between 1.3 and 2.5) among the 4 parameters tested. Within the same particle size (grit), the order of measurement is Pq > Pa > Ra.

Table 3 shows the surface texture Ra, Rs, Pa, and Pq values of 3 sliced food surfaces under 3 sample conditions, that is, fresh-cut, glutaraldehyde-fixed, and critical point dried treatments. The line segment roughness (Ra) data showed the sliced food surface roughness ranging from 3.31 (cheese) to 5.19 (ham) to 8.04 (salami) and the Rs values from 2.09 to 3.07 to 4.17, respectively. In general, the Rs values showed a narrower range (1.58 to 6.32) compared to Ra (2.04 to 10.39) in this study, which might be due to the influence of imaging conditions and the computer algorithm (Wendt and others 2002). Pohl and Stella (2002) also pointed out that the difficulty to directly determine the real surface by conventional techniques has reduced the applicability of this parameter. However, this may not be true with the current computer technology and other advantages. The order of measured roughness for fresh-cut food samples is cheese (low), ham, and salami (high), which is consistent with the observations. After the cross-linking and critical point drying treatments, the roughness changed and became inconsistent. The surface roughness of cheese increased in the order from dried to fresh-cut to glutaraldehyde-treated. The roughness order (low
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to high) for ham was glutaraldehyde-treated, dried, and fresh-cut, except Rs. However, the roughness order of salami was fresh-cut, glutaraldehyde-treated, and dried, except Rs. The roughness values of dried salami also had a much higher standard deviation (±33% of mean), which could be due to its localized high fat content and other particulates (spices) added to the product. The Ra of salami also showed less sensitivity (between 8.04 and 10.39) compared with values of the ROI roughness, Rs, Pa, and Pq. Therefore, any food treatment or process might have a different degree of impact on surface roughness which would be difficult to predict, especially for complicated food compositions with chemical and physical reactions involved during processing and production. The roughness order of the food surface (treated or nontreated) is Pq > Pa > Ra > Rs, except for glutaraldehyde-treated ham, where Pq > Pa > Rs > Ra.

Figure 4A to 4C illustrate the 2-D images of fresh-cut cheese, ham, and salami, respectively. They indicate the similarity in structure features (in 2-D pattern) between the soft food surfaces and the rigid sandpaper surfaces. Figure 5A to 5C demonstrate the 3-D surface topographies of fresh-cut cheese, ham, and salami, respectively, where the darker areas indicate deeper valleys and lighter areas represent higher peaks. Salami (Figure 5C) again displayed the roughest surface texture.

The use of CLSM to investigate and measure 3-D structure, especially the surface texture of sliced food, has been demonstrated with surface roughness in terms of Ra, Rs, Pa, and Pq. Parameters such
as skewness and kurtosis of surface height distributions, which require other software, were not evaluated. Bouchon and others (2003) showed that no significant difference of these 2 parameters in 3 distinct restructured fried potato chip surfaces. As mentioned previously, lack of standard instrument settings to achieve reliable measurements is one of the barriers to broaden the applications of CLSM for roughness measurement. This study provides a method to verify and calibrate the instrument set-up. With surface roughness far beyond the range of current sandpaper dimensions, other size standards might be needed to validate the CLSM results. Other factors, for example, sharp edge and tilted surface, which might impact the data acquisition and measurement results, remain to be investigated.

Although atomic force microscopy (AFM) has been widely used to investigate the food component/structure at the micro- and nanometer scales (Yang and others 2007), CLSM has several advantages over AFM in food science applications, including scanning speed and scanning size. Other advantages of using CLSM in surface analyses include (1) significantly enhanced lateral (x, y) and axial (z) resolution for 3-D image sectioning; (2) improved image contrast due to the confocal aperture, which reduces the amount of light from above and below the focal plane. Chen and others (2006) have detailed the merits of CLSM applications in foods. Moreover, thanks to the recent advances in computing and data handling, surface texture-related studies have become more feasible. The CLSM might be applied to many other food surface texture evaluations, for example, avian eggshells, and other safety-sensitive agricultural surfaces, which may have significant impact on the surface-cleaning process efficiency per microbial safety concern.

**Conclusions**

Surface topography characterization using the reflective CLSM image with line segment and area (ROI) data analyses can be used as a metrology tool for surface roughness of food matrices. The settings of CLSM instrument can be reproducibly verified with sandpaper particle sizes and applied to the sandpaper surfaces, then to sliced foods for quantitative evaluation of the surface roughness. This report describes one approach for using the reflective CLSM to quantitatively evaluate processed food surfaces. Due to the increased availability and affordable cost, CLSM is becoming...
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Figure 5—3-D surface topographies of fresh-cut cheese (A), ham (B), and salami (C).

a more popular tool to characterize the food surface texture, and this may significantly impact engineering for food appearance, flavor release, process design, and/or microbial transfer.

Acknowledgment
We thank Paul Pierlott, ERRC/ARS/USDA, Wyndmoor, Pa., for his expert assistance with image processing and preparation of figures for 3-D topography and Dr. Vladimir Zhukarev of Leica Microsystems for advice and information concerning imaging.

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