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Surface topography of heat-set whey protein gels by confocal laser scanning microscopy

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Abstract

Extensive research works have been carried out in investigating the microstructure of heat-set whey protein gels and their fractal nature, but little has been done on the surface studies of these systems due to the lack of suitable technique for surface characterization of wet and deformable food gels. This work intended to explore the possibility of applying confocal laser scanning microscope (CLSM) for surface characterization of such delicate systems. Surfaces of heat-set whey protein gels (14 wt%) have been investigated with and without the presence of salt (0 and 200 mM NaCl). High quality surface images across *X–Z* plane were obtained. These images were further analysed for their surface roughness, periodic length scale, and fractal nature. The roughness of these surfaces was quantified in terms of root-mean-square surface roughness (R_q) and arithmetic surface roughness (R_a). It was found that the protein gel without salt addition had a very smooth surface with R_q of 0.20 and 0.18 μm, respectively, but the gel containing 200 mM NaCl had a much rougher surface with large R_q and R_a (2.39 and 1.91 μm, respectively). The fractal nature of the surface was also revealed for this gel and a fractal dimension of 1.15 was obtained.
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1. Introduction

Surface properties such as surface roughness and surface wetness strongly influence the visual and sensorial quality of food products (Malone, Appelqvist, & Norton, 2003). Consumers always have the preference of food products, which have appealing surface appearance and texture. Although the importance of surface properties of foodstuff has been well recognized, the creation and characterization of surface texture are still poorly understood. Little progress has been made so far on the surface quantification of food materials despite of various techniques that have been made available for surface researches of non-food materials. Very recently, surface friction measurement method has been successfully applied in authors' group for the characterization of surface properties of heat-set whey protein gels (Chen, Moschakis, & Nelson, 2004). It was found that, by analysing the speed- and load-dependence of surface friction, the surface roughness and wetness of protein gels could be easily distinguished. This work will further explore the possibility of quantitative characterization of surface geometry of these protein gels based on the images from confocal laser scanning microscope.

Surface geometry is by nature a three-dimensional feature. In theory, any measurement of two-dimensional profiles or sections cannot give a complete description of the real surface topography features. However, in practice, two-dimensional measurements are still generally acceptable on the assumption that the surface is isotropic, that is, the surface has the same topography features across the *X–Y* plane. Based on the analysis of the profile of a surface, characteristic features of the surface can be quantitatively described using a number of statistical parameters, such as root-mean-square roughness (R_q) and average (or arithmetic) roughness (R_a). Root-mean-square roughness is defined as the square root of the mean of the height square

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deviations from the mean and is probably the most commonly used surface roughness parameter

$$R_q = \sqrt{\frac{\sum_{i=1}^{N}(z_i - z_a)^2}{N}}, \quad (1)$$

where z_i is the surface height at each measuring point, N is the total number of measuring points, and z_a is the mean height of the surface profile and is defined as

$$z_a = \frac{1}{N}\sum_{i=1}^{N} z_i. \quad (2)$$

Average (or arithmetic) roughness is defined as the average of deviation of surface from its baseline and is also commonly used for surface geometry quantification:

$$R_a = \frac{1}{N}\sum_{i=1}^{N} |z_i - z_a|. \quad (3)$$

Both root-mean-square roughness and average roughness gave a statistical value of surface roughness and were found effective in describing and differentiating surface topography of various materials (Maksumov, Vidu, Palazoglu, & Stroeve, 2004; Tay, Sikdar, & Mannan, 2002). Parameters such as peak-to-valley height, skewness of surface height distribution, and kurtosis of surface height distribution are also used for surface geometry characterization.

There has been great achievement in developing techniques for surface characterization during last few decades (Myshkin, Grigoriev, Chizhik, Choi, & Petrokovets, 2003). Tribometry and contact profilometry are the classical techniques widely used for the surface characterization of solid materials (Chappard et al., 2003; Felder & Samper, 1994; Luengo, Tsuchiya, Heuberger, & Israelachvili, 1997; Tay et al., 2002). Optical Microscopy is a convenient technique for surface observation of almost any material, but application of optical lenses means that the resolution of normal optical microscope can only reach micrometer length scale. Surface scanning microscopy such as atomic force microscopy (AFM) is probably the most powerful technique so far for surface imaging and quantification. Since its introduction less than two decades ago (Binning, Quate, & Gerber, 1986), AFM has been widely used for high resolution profiling of surface morphology and nanostructure of various materials (Chakrapani, Mitchell, van Winkle, & Rikvold, 2003; Jalili & Lasminarayana, 2004; Jandt, 2001; Morris, Kirby, & Gunning, 1999; Pang, Baba-Kishi, & Patel, 2000). High resolution and capability of nanoscale surface profiling are unmatchable advantages of this technique. Scanning electron microscopy (SEM) is another powerful technique for high quality surface imaging and profiling of various materials (Brooks & McGill, 1994; Chappard et al., 2003; Huang, Li, Shen, Zhu, & Xu, 2002; Vansteenkiste, Davies, Roberts, Tendler, & Williams, 1998). With the help of ever advancing software, SEM can now produce high quality three-dimensional surface images and perform complex surface quantification. Other established technique for surface characterization include methods based on the specularly reflected light of surfaces such as: Fibre Optic Reflectometer (Silvennoinen et al., 1993), Glossy Meter (Huang et al., 2002), surface glistening points method (Lu, Koenderink, & Kappers, 1999; Quevedo & Aguilera, 2004) and methods based on surface contact such as contact angel measurement (Meiron, Marmur, & Saguy, 2004).

While the above mentioned techniques have their unique advantages for the characterization of certain type surfaces and materials, they all show great limitation for surface imaging and characterization of protein gels and other food materials. Surface deformability and the existence of surface moisture of such food materials are the main problems for the application of these techniques. For example, damage of a delicate surface could be easily caused by the tapping probe of an AFM and other techniques, which use a contacting surface-probe. Surface moisture of protein gels and other wet food material is the biggest problem for the application of SEM technique to wet surfaces. In these cases, surface dehydration would be essential, but surface distortion would almost be inevitable. Environmental scanning electron microscopy (ESEM) was further developed for surface imaging of wet materials without the need of a full removal of surface moisture. In reality, however, the quality of surface image will have to be compromised for high moisture containing systems such as protein gels whose water content could be as high as 99%.

In addition to these limitation and drawbacks, there is a non-stated assumption for these surface characterization techniques: the surface asperities are cone-shaped or column-shaped, or that the tips of the asperities are at least no larger than their bases. However, if the bases of asperities are smaller than their tips or bodies, such as diamond-shaped, mushroom-shaped and upside down cone-shaped asperities, surface profiles based on the above techniques could be seriously misleading. This is because the void space beneath the head of a mushroom-shaped asperity, for example, is simply undetectable by either a surface contacting probe or by surface reflected light. It is probably true that asperities of most engineered surfaces are cone-shaped or column-shaped. However, many naturally grown surfaces may not necessarily follow this rule. For example, particulate gels have networks of particle strands growing with a particular pattern in certain directions (Dickinson, 1994, 1995; Doi, 1993; Stading & Hermansson, 1991). Therefore, diamond-shaped, mushroomed-shaped, or upside down cone-shaped surface asperities could be highly possible in aggregated particle gels.

Confocal laser scanning microscope (CLSM) could provide a solution to the above problems. As a non-conventional light microscope, CLSM technique has a number of advantages for microstructure observation of foodstuff and colloidal systems. With this technique, one can obtain images of the microstructure of the surface or an

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