Ptychographic X-ray computed tomography of extended colloidal networks in food emulsions

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ABSTRACT

As a main structural level in colloidal food materials, extended colloidal networks are important for texture and rheology. By obtaining the 3D microstructure of the network, macroscopic mechanical properties of the material can be inferred. However, this approach is hampered by the lack of suitable non-destructive 3D imaging techniques with submicron resolution.

We present results of quantitative ptychographic X-ray computed tomography applied to a palm kernel oil based oil-in-water emulsion. The measurements were carried out at ambient pressure and temperature. The 3D structure of the extended colloidal network of fat globules was obtained with a resolution of around 300 nm. Through image analysis of the network structure, the fat globule size distribution was computed and compared to previous findings. In further support, the reconstructed electron density values were within 4% of reference values.

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1. Introduction

Extended colloidal networks constitute one of the main structural elements in multi-phase food materials such as butter, chocolate, cream cheese, whipped creams, ice cream, cheese and yogurt. Focus has been directed toward establishing the relationship between the structure of such networks on one side, and the macroscopic mechanical properties and sensorial textural properties on the other side (Heertje, 2014; Narine & Marangoni, 1999b). On a qualitative level, correlations have been established between microscopic observations of loose networks and low modulus or relationship between a more dense network and a higher modulus (see for example Buldo & Wiking, 2011; Kaufmann, Andersen, & Wiking, 2012; Wiking, De Graef, Rasmussen, & Dewettinck, 2009). On a more quantitative level, a fractal description has been employed for inorganic colloidal networks (Shih, Shih, Kim, Liu, & Aksay, 1990), for protein systems (Stading, Langton, & Hermansson, 1993; Vreeker, Hoekstra, den Boer, & Agterof, 1992) and fat systems (Marangoni et al., 2012; Marangoni & Hartel, 1998; Narine & Marangoni, 1999a) leading to a quantitative relationship between structure and mechanical properties.

The experimental study of such networks has traditionally been based on light microscopy such as polarized light microscopy (PLS) and confocal laser scanning microscopy (CLSM). From the microscopy measurements, the network properties have for the most been analyzed using 2D slices. An extension to 3D imaging can be done by forming 3D image stacks from consecutive micrography at different depths in the food product. In this way, Litwinenko used transmitted PLS and image deconvolution to study the fractal properties of a fat crystal network in two and three dimensions (Litwinenko, 2004). Similarly, CLSM can be applied as an 3D imaging method within food science. However, the obtained stacks are limited by the optical system and the laser penetration depth to some tens of microns from the top surface (Dürrenberger, Handschin, Conde-Petit, & Escher, 2001). Furthermore, although a sub-micron spatial resolution is possible, scattering in the sample may limit the resolution along the vertical axis to the micron range (Fredrich, 1999). In addition, the need of staining in CLSM may introduce artifacts and limit the in-situ applicability (Dürrenberger et al., 2001). To our knowledge CLSM has not been used to image the 3D structure of colloidal networks in food materials.

In recent years, X-ray phase-contrast computed tomography (CT) has emerged at synchrotron facilities as a non-destructive 3D imaging modality, and has been successfully applied to study the microstructure of a range of food products (Falcone et al., 2004; Jensen et al., 2011; Miklos, Nielsen, Einarsdóttir, Feidenhans'l, & Lametsch, 2015; Verboven et al., 2008). One of the most recent techniques for obtaining the X-ray phase-contrast modality is ptychographic X-ray computed tomography (PXCT) (Dierolf et al., 2010). PXCT is a 3D nano-imaging technique that offers a spatial resolution in the 100 nm range. Unlike traditional X-ray microscopy, the spatial resolution is not dependent on objective lenses. Instead, spatial information is retrieved from the recorded diffraction of a coherent X-ray beam, and the spatial resolution is only limited by the angular spread of the scattered intensity. In addition, PXCT provides quantitative information by reconstructing the full 3D electron density distribution of the specimen (Diaz et al., 2012; Thibault, Guizar-Sicairos, & Menzel, 2014). The technique is well-suited for in-situ measurements as it allows for sample environments at room temperature and ambient pressure. Previously, it has successfully been applied for an in-situ study of water uptake in a single silk fiber (Esmaeili et al., 2013). Altogether, PXCT is a promising candidate for 3D imaging of extended networks in food products.

As a model system for studying extended colloidal networks, a palm kernel oil (PKO) based oil-in-water emulsion is presented. These PKO emulsions are used as whippable creams for decorations of cakes where the fat globule network formation is important. Design of emulsions for whipping relies on tuning the propensity of partial coalescence of the oil droplets. Initially PKO emulsions are normally liquid, and first upon whipping the material is transformed to a foam of rather high viscosity and stability. However, too high propensity for partial coalescence can lead to product flaws such as solidification of the liquid emulsion to solid pastes during transport. As an example of a product flaw, a PKO emulsion exhibited pre-whipping solidification upon addition of two different combinations of lactic acid ester of monoglyceride (LACTEM) and unsaturated monoglyceride (GMU) (Munk & Andersen, 2015; Munk et al., 2013). In these two systems, 2D CLSM micrographs of the lipid phase revealed large irregular aggregates and formation of extended networks of fat globules. In addition, increased hardness and viscoelastic modulus were observed. The added emulsifiers are believed to induce partial coalescence of the fat globules and transform the emulsion spontaneously from liquid to semi-solid (Munk & Andersen, 2015). However, due to strong multiple scattering of the laser light, the 3D structure of the network could not be determined using CLSM.

Thus, the exact extend and composition of the network of fat globules in 3D are still unknown. In addition, exactly how the water and lipid phases are located remain to be directly observed. In this study, a PKO emulsion with two combinations of LACTEM and GMU emulsifiers are measured with PXCT and compared to 2D CLSM micrographs. The 3D structure of both water and lipid phase as well as the quantitative electron density values are investigated.

2. The X-ray phase-contrast modality

The type of image contrast acquired in X-ray tomograms depends on the interaction between the X-rays and matter. Both refraction and absorption of X-rays in matter are given by the full complex index of refraction (Als-Nielsen & McMorrow, 2001)

$$n(\mathbf{r}) = 1 - \delta(\mathbf{r}) + i\beta(\mathbf{r}), \tag{1}$$

where the real part δ accounts for the refraction and the imaginary part β for the absorption. In X-ray phase-contrast techniques such as PXCT, the 3D distribution of $\delta(\mathbf{r})$ is reconstructed in the tomogram. Thus, the gray levels in the resulting images are due to the spatial variations of $\delta(\mathbf{r})$ in the material. These can be related to the electron density $\rho_e(\mathbf{r})$ as

$$\rho_e(\mathbf{r}) = \frac{2\pi\delta(\mathbf{r})}{r_0\lambda^2}, \tag{2}$$

where r_0 is the Thomson scattering length and λ the X-ray wavelength. For materials with known atomic composition, values of ρ_e obtained by PXCT can be compared to calculated values. For mixtures of materials of different weight-percentages w_j, the total electron density can be calculated as

$$\rho_e = N_A\rho_m \sum_j w_j \frac{Z_j}{M_j} \tag{3}$$

where N_A is Avogadro's constant, ρ_m is the mass density and Z_j and M_j are the number of electrons and the molar mass of the jth material, respectively.

3. Material and methods

3.1. Materials

Emulsifiers were of commercial food grade and all were provided by Palsgaard A/S (Juelsminde, Denmark): Lactic acid ester of monoglyceride (LACTEM) made from fully hydrogenated palm oil and rape-seed oil (C16:0 and C18:0 >97% of fatty acids); unsaturated monoglycerides (GMU) made from sunflower oil (C18:1 >81%). The stabilizer mixture (Palsgaard A/S, Juelsminde, Denmark) contained microcrystalline cellulose (MCC), sodium carboxymethylcellulose (CMC) and disodium phosphate. Hydrogenated palm kernel oil (PKO) was obtained from AAK (Karlshamn, Sweden), sodium caseinate from DMV International (Veghel, The Netherlands) and sugar from Nordic Sugar (Nakskov, Denmark). Fatty acid composition of the PKO has previously been determined (Munk & Andersen, 2015).

3.1.1. Emulsion blend preparation

Sodium caseinate (0.6 wt.%), stabilizer mixture (0.6 wt.%) and sugar (10 wt.%) were dispersed in water under continuous stirring and put aside for 4 h to hydrate proteins. Melted PKO (25 wt.%), either LACTEM (0.55 wt.%) alone or with GMU (0.15 wt.%) were mixed with the water phase, and the mixture was heated to 80 °C. A pre-emulsion was obtained by mixing with a high-shear blender (Ultra-Turrax, IKA, NC, USA) for approximately 20 s. Homogenization was subsequently carried out on a two-stage high-pressure valve homogenizer (PandaPlus 2000. GEA Niro Soavi, Parma, Italy) at 150/50 bar followed by cooling in a turbular heat exchanger to 30 °C. Immediately afterwards, small samples of the emulsions were prepared in micropipettes using a syringe. Subsequently, all emulsion samples were stored at 5 °C.

3.1.2. Micropipette preparation

The micropipettes were prepared from thin wall borosilicate capillaries with filament (Harvard Apparatus UK, Cambridge, UK) by using a micropipette puller (Sutter Instrument, CA, USA). The capillaries were pulled from the original 0.94 mm to an inner diameter at the tip in the range from 15 to 20 μm. Prior to measurements, the micropipettes were mounted on custom-made tips.

3.2. Confocal laser scanning microscopy

Micrographs of the lipid phase in the emulsion were obtained by an inverse confocal laser scanning microscope (Leica TCS SP5, Heidelberg, Germany). Total concentration of 1 ppm BODIPY

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