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# Air bubbles in fibrous caseinate gels investigated by neutron refraction, X-ray tomography and refractive microscope

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ABSTRACT

Fibrous protein gels have the potential to form the basis for the next-generation of meat analogue. It is suggested that fibre anisotropy is related to air bubbles present in the gel. Given the complexity and heterogeneity of the samples, several quantitative techniques are needed to ensure a comprehensive understanding of the air bubbles. We performed neutron refraction experiments to study the size and shape of the air bubbles in three calcium caseinate samples containing different $H_2O$ to $D_2O$ ratios. Refractive microscopy and X-ray tomography (XRT) analysis were done on the same samples to provide complementary information. The deformation degree and average width of the air bubbles were obtained from both the XRT and neutron refraction experiment. A neutron refraction model calculates the average area and volume of a single air bubble, which correspond to the largest area and volume fractions of all the air bubbles from the XRT analysis. Additionally, we found that the $H_2O$ to $D_2O$ ratios in the sample largely influence the size, number distribution and deformation degree of the air bubbles. The neutron refraction technique can be a simple and complementary method to help understanding the role of air bubbles in the meat analogue.



## 1. Introduction

Many food products can be seen as composite materials whose textures are determined synergistically by the fillers (dispersed phase) and the matrix (continuous phase) (van Aken, Oliver, & Scholten, 2015; van der Sman, 2016). For example, the viscoelastic properties of a dough change after the inclusion of air (Dobraszczyk & Morgenstern, 2003); the hardness of a cheese increases with decreasing fat content (Bryant, Ustunol, & Steffe, 1995), and the rheological and sensory properties of a chocolate rely on the subtle balance between sugar, cocoa butter and cocoa powder (Afoakwa, Paterson, & Fowler, 2007). Characterizing the microstructure of the dispersed phase is crucial to understanding the macroscopic properties of the food product. (see Fig. 7)

An interesting composite food material is a fibrous protein gel made of calcium caseinate (Grabowska, van der Goot, & Boom, 2012; Manski et al., 2007a). Since the fibrous structure resembles real meat, calcium caseinate is considered as a promising candidate for the next-generation meat analogue (Manski et al., 2007b). Air is the only dispersed phase in such a fibrous protein gel. It has always been present in different types of meat analogues (Krintiras, Diaz, Van der Goot, Stankiewicz, & Stefanidis, 2016; Manski et al., 2007c), but it was not until recent that its contribution to the fibres' mechanical properties was brought up (Dekkers, Hamoen, Boom, & van der Goot, 2018). The air bubbles are elongated along the shear rate direction and its maximum length corresponds to the highest anisotropy index of the fibres. This suggests that characterising the air bubbles can provide complementary information on the anisotropy of the fibres. However, not many techniques are available to characterize them.

X-ray computed tomography (XRT) is a common and non-destructive technique to study the size, shape and amount of air in composite materials (Das et al., 2016; Kuang, Ying, Ranieri, & Sansalone, 2015; van Dalen, Blonk, van Aalst, & Hendriks, 2003). The contrast in XRT comes from the absorption difference between the protein matrix and the air. XRT has been applied to demonstrate how gelatin peptide fortified the microstructure of frozen mousses against the freeze-thaw cycle (Duquenne et al., 2016), to evaluate the performance of different gluten (Bellido,

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Scanlon, Page, & Hallgrimsson, 2006) or gluten-free dough recipes (Demirkesen et al., 2014), and to support a new preparation method for making a soy-protein based hydrogel (Dekkers et al., 2018; Guo et al., 2013). As powerful as XRT can be, a composite food system is inherently heterogeneous and complex (Donald, 2004), so no single remedy is sufficient to provide a complete view of the sample. The drawbacks of XRT are the large dataset, possible imaging artifacts, and the treatment of the raw data can be dependent on the operators (Cnudde & Boone, 2013; Maire & Withers, 2014; Schoeman, Williams, du Plessis, & Manley, 2016). To ensure a comprehensive and unbiased understanding of the sample, we need several quantitative approaches to characterize the air bubbles.

Neutron refraction is another suitable technique to study the air bubbles. It is performed by the Spin-Echo Small Angle Neutron Scattering (SESANS) instrument (Plomp, Barker, De Haan, Bouwman, & van Well, 2007). The SESANS instrument measures the change in neutron direction. This results in a decrease of the polarisation of neutron beam. When the size of an object is small (e.g., up to a few micrometers), the change in polarisation is contributed by the scattering of the object. The scattering depends on the dimension of the scattering particle, volume fraction and scattering length density contract. Thus, the size information is readily present in the measured depolarisation curve (Berk & Hardman-Rhyne, 1988; de Haan, Plomp, & van Well, 2007a). It was applied to quantify the microstructure of globular protein gels (Ersch et al., 2016), to explain the mechanical properties of cross-linked casein micelles (Nieuwland, Bouwman, Bennink, Silletti, & de Jongh, 2015), and to compare the gel structure between curdling and acidified milk (Tromp & Bouwman, 2007). When the size of an object is large (e.g., over tens of micrometers), neutrons passing through the object will be rather refracted than scattered (Berk & Hardman-Rhyne, 1988; de Haan, Plomp & van Well, 2007b). The amount of refraction depends on the geometry of the object instead of the size, so the size information is not directly visible in the detected signal. The neutron refractive index difference between the object and its surrounding matrix determines the contrast. Neutron refraction has been less explored than neutron scattering. To our knowledge, it has only been used in food-related research once, to characterize the number of layers in a fibrous structure made of soy protein isolate and gluten (Krintiras, Göbel, Bouwman, Van Der Goot, & Stefanidis, 2014). The fibre was approximated as infinitely long cylinders with a spread in orientation. However, the geometry of a fibre is very different from that of an elongated air bubble. As a result, another model is needed so that the information obtained from the neutron refraction technique is comparable to the others.

In this study, we demonstrated multiple techniques: neutron refraction combined with XRT and refractive microscope add more certainty to a comprehensive understanding of the air bubbles dispersed in the fibrous calcium caseinate gel. To show the sensitivity of the contrast, we prepared three samples using 100% $H_2O$, 50%$v/v$ $H_2O$+50%$v/v$ $D_2O$ and 100% $D_2O$. An improved model based on the microscopy images was proposed to fit the neutron refraction data. Parameters such as deformation degree, average width and orientation of the air bubbles can be extracted from the model, which agrees with the XRT results.

## 2. Material and methods

### 2.1. Materials

Roller dried calcium caseinate powder was provided by DMV International, Veghel, The Netherlands. The calcium caseinate powder contains 90%*wt* protein and 1.2%*wt* calcium according to the manufacturer's specification. Heavy water (deuterium oxide, $D_2O$, $\rho = 1.107$g/mL) with 99.9% deuterium was used (Sigma-Aldrich, Canada).

### 2.2. Preparation of the structure

An in-house developed shear device with gap angle 2.5° (Wageningen University, The Netherlands) was used to obtain the anisotropic calcium caseinate samples. The shear device is depicted and described in detail in (Manski et al., 2007a; van der Zalm, Berghout, van der Goot, & Boom, 2012). A heating and a cooling water bath were connected to the rotating and stationary cone to control the temperature. Protein premixes were prepared by manually mixing 30 g calcium caseinate powder with 70 g demi-water (100% $H_2O$), 35 g demi-water+38.9 g heavy water (50%$v/v$ $H_2O$+50%$v/v$ $D_2O$) or 77.7 g heavy water (100% $D_2O$). The premixes were transferred to the pre-heated (50°C) shearing device and processed with a rotating speed of 150 rpm for 5 min. After processing, the samples were cooled to 4°C in 10 min before removal from the device. They were stored at −20°C for further analysis. Two sets of samples were made for duplicate measurements.

### 2.3. Neutron refraction

The effect of the refracted neutron is measured by the SESANS instrument at the neutron source in Delft University of Technology, the Netherlands. The principle of this instrument involves the Larmor precession of a polarised neutron beam in a magnetic field, where the angular deviations of the neutron trajectory due to scattering or refraction are encoded by the measured polarisation $P$ (Bouwman et al., 2004; Rekveldt et al., 2002). When the air bubble is small (e.g., up to a few $\mu$m), only elastic scattering contributes to the change in polarisation. However, when the air bubble is large (e.g., over tens of $\mu$m), refraction of the neutrons becomes the dominant cause of change in polarisation. The transition from scattering to refraction is continuous. For the cross-over region, we need more advanced theory, such as the phase-object approximation (de Haan et al., 2007a; 2007b), to calculate the angle distribution function. Nevertheless, we can estimate a size limit for this transition. It is calculated by the phase change of the neutron wave function when going through the air and protein interface. When the phase change is of the order of $\pi$, refraction becomes dominant (de Haan et al., 2007b). The neutron wave phase change $\eta$ is calculated as

$$\eta = \left|\frac{4\pi\delta R}{\lambda}\right| \tag{1}$$

where $R$ is the radius of a sphere, $\lambda$ is the wavelength of the neutron, the SESANS instrument in Delft uses thermal neutrons with a wavelength of 2.1±0.1A°, $\delta$ is the difference between the neutron refractive index and 1.

$$\delta = \frac{\Delta SLD \cdot \lambda^2}{2\pi} \tag{2}$$

ΔSLD is the scattering length density contrast between the protein matrix and the air bubbles. For the samples made of 100% $D_2O$, this number is $5.02 \times 10^{14}\mathrm{m}^{-2}$, for the sample made of 50%$v/v$ $H_2O$+50%$v/v$ $D_2O$, $\Delta SLD = 2.74 \times 10^{14}\mathrm{m}^{-2}$, and ΔSLD equals to $0.208 \times 10^{14}\mathrm{m}^{-2}$ for the sample made of 100% $H_2O$. Knowing $\lambda$ and $\delta$, we calculated the minimum air bubble size, and can be sure that refraction dominates the measured signal when the size is around 30$\mu$m.