



## Analysis of micro-structure in raw and heat treated meat emulsions from multimodal X-ray microtomography



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### ABSTRACT

This study presents a novel non-destructive X-ray technique for analyzing meat emulsions before and after heat treatment. The method is based on X-ray grating-interferometry where three complementary imaging modalities are obtained simultaneously measuring the absorption, refraction and scattering properties of the sample. Enhanced contrast capabilities of this X-ray technique makes studies on materials with similar attenuation properties possible. The emulsion samples were imaged both in a raw and cooked state. Additionally, different fat types were used in the emulsions in order to compare microstructural differences when either pork fat or sunflower oil was added. From the reconstructed tomograms the different constituents in the emulsions were segmented using a multivariate segmentation method. From this, a quantitative analysis was performed between the different samples, determining properties such as percent object volumes, porosity, average structure thickness and cooking loss. The grating-based X-ray technique and multivariate segmentation made the analysis of the microstructure possible which further gives insight to how both heat treatment, and the use of different lipid types, affect the final protein network quality.

**Industrial relevance:** Meat emulsions have previously been thoroughly studied, and the use of various fat substitutes and protein stabilizers has been investigated. The grating-based multimodal X-ray tomography method presented here is a feasible method to investigate the microstructural changes induced by heat treatment. It provides high-resolution three dimensional spatial information and in contrast to 2D imaging methods, quantitative parameters can be extracted by image analysis for the entire sample volume. Additionally, the non-destructive method allows for imaging the same sample before and after cooking.

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### 1. Introduction

The most important functional characteristics in comminuted meat products are the gel-forming abilities of the myofibrillar proteins. During comminution, salt-soluble myofibrillar proteins are extracted that, when heated, create a dense protein network referred to as gel (Tornberg, 2005). Some of the solubilized proteins will emulsify the added fat by forming an interfacial protein film around the fat globules, which are further stabilized by the protein gel (Barbut, 1995; Wu, Xiong, Chen, Tang, & Zhou, 2009). The fat globules act as fillers, reducing the porosity and increasing the stability of the gel. Differences in the physicochemical properties of saturated and unsaturated lipids, i.e. emulsification properties and physical state, will affect the distribution of fat and the influence on the gel stability and thereby the quality of the final product. Due to health aspects, substitution of animal fat with vegetable oil has generated interest in the meat processing industry

(Wood et al., 2004). The lower melting point of the vegetable oil facilitates an even distribution of small oil droplets in the meat batter leading to formation of a homogeneous gel structure. However, the higher mobility of the oil compared to the solid animal fat is a challenge. The coalescence of oil droplets may lead to channel formation in the protein network facilitating moisture transportation during heat treatment which can be observed as increased cooking losses (Barbut, 1995).

Studies on the quality of meat emulsions rely on a variety of measurements. These include determination of pH values, cooking loss, color composition, texture profiles, apparent viscosity, and emulsion stability (Choi et al., 2009, 2010; Gordon & Barbut, 1991; Shao, Zou, Xu, Wu, & Zhou, 2011). Current imaging techniques used to analyze the quality of meat emulsions have mainly focused on two dimensional measurements from either scanning- and transmission electron microscopy (Álvarez et al., 2012; Totosaus & Pérez-Chabela, 2009), light micrographs (Álvarez & Barbut, 2013; Youssef & Barbut, 2009, 2010), or confocal laser scanning microscopy (Sorapukdee, Kongtasorn, Benjakul, & Visessanguan, 2012). Due to the similar attenuation properties of the soft materials in meat emulsions, the use of X-ray microcomputed

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tomography ( $\mu$ CT) has been limited. In Santos-Garcés et al. (2012) a feasibility study of X-ray  $\mu$ CT for microstructure analysis of fermented sausages demonstrated that absorption tomography provided contrast between meat, fat and air holes. Although this  $\mu$ CT analysis identified fat particles and air holes, the technique was not accurate enough to distinguish between pork lean and fat when these constituents were emulsified. Novel X-ray techniques based on grating-interferometry provide new imaging modalities that can be obtained simultaneously with absorption tomography (Bech et al., 2010). These modalities, phase contrast and dark-field imaging, measure the electron density and the diffusion length of the sample. Enhanced contrast capabilities of this X-ray technique makes studies on materials with similar attenuation properties, such as soft tissue, possible. Previous studies have demonstrated superior contrast with X-ray phase-contrast CT compared to conventional CT in a study of pork rind and fat (Jensen et al., 2011), and demonstrated the potential for improved segmentation when using multivariate analysis by combining conventional CT with phase-contrast CT for bivariate segmentation of a piece of pork back fat and a piece of beef muscle tissue (Nielsen et al., 2012).

In this paper, the novel X-ray technique is used to investigate the differences in microstructures of meat emulsions in three dimensions. Such analysis allows for determining structural parameters of the entire sample instead of inferring from partial information obtained by two dimensional imaging techniques. The information obtained from a three dimensional analysis is believed to further increase the understanding of emulsion microstructure. Additionally, the non-destructive technique offers the possibility to study the same sample in both raw and cooked condition. The samples used were raw and heat treated meat emulsions (10% protein, 25% fat, 60% moisture) prepared with either pork fat (lard) or sunflower oil. Absorption, phase contrast and dark-field tomograms were obtained at a synchrotron facility using a grating interferometer. From the reconstructed tomograms the different constituents in the emulsions were segmented using a multivariate segmentation method. A quantitative analysis was performed by measuring geometrical parameters in order to determine the microstructural differences of the emulsions when using lard or sunflower oil and also the effect heat treatment has on the emulsion quality.

## 2. Materials and methods

### 2.1. X-ray modalities

In Fig. 1 the three types of physical interactions – absorption, refraction and scattering – obtained from the absorption, phase-contrast and dark-field imaging modalities of grating-based interferometry are illustrated. The effect on an incoming Gaussian shaped beam profile (black) is depicted when elements with different physical properties are measured. The profiles shown in color represent what is recorded when a material is present. In green, the effect from an absorptive material is shown to attenuate the beam, while in blue, the effect of a refractive material is seen to cause a transverse shift in the position of the beam profile. Lastly, the small-angle scattering from a material with ordered micro-structures causes the beam profile, here shown in red, to broaden. By separating the attenuation, transverse shift and broadening of the beam, it is thus possible to measure three complementary imaging modalities. For further details on the X-ray modalities, the reader is referred to Bech, Jensen, et al. (2010) and Pfeiffer (2012).

### 2.2. Grating-based interferometry

One method to separate the three X-ray interactions is grating-based imaging (GBI), which relies on an X-ray interferometer consisting of periodic gratings for measurements. A schematic of a setup for GBI is shown in Fig. 2. Grating G1 produces a periodic intensity modulation, consisting of periodic fringes, transverse to the beam direction. The change in position, mean value and amplitude of the periodic fringes

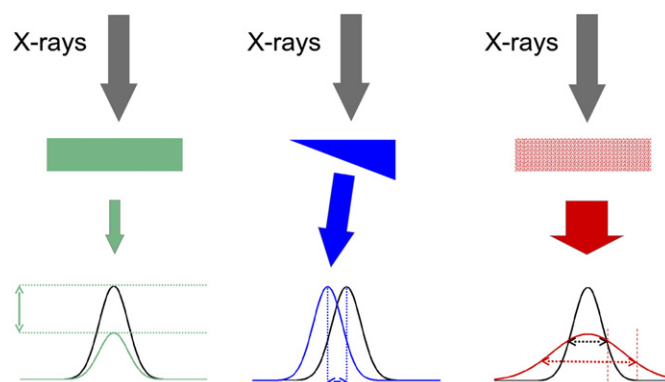


Fig. 1. The incoming X-ray beam changes when a sample is present. The effect on the beam from an absorptive material is shown in green, a refractive material in blue and a material with a homogeneous distribution of micro-structures in red.

can be probed using a second grating, G2, by physically moving one of the gratings in several steps, acquiring a projection image between each movement of the grating. From the same series of scans, both the absorption, refraction and small-angle scattering can be extracted giving an inherent pixel correspondence. Tomograms are then created for each modality using filtered back-projection. This results in absorption, phase-contrast and dark-field image volumes, measuring the attenuation length, electron density and the linear diffusion coefficient of the sample, respectively (Bech et al., 2010; Weitkamp, David, Kottler, Bunk, & Pfeiffer, 2006). GBI using synchrotron sources was first demonstrated in the beginning of the 2000s (David, Nohammer, Solak, & Ziegler, 2002; Momose, 2003; Weitkamp et al., 2005), and later adapted to laboratory-based setups (Pfeiffer, Weitkamp, Bunk, & David, 2006). The method can be applied using polychromatic sources but a certain degree of spatial coherence is needed. In a laboratory setup, spatial coherence can be achieved either by using a microfocus source or by using a third grating G0, which acts as an array of line sources for use with source sizes up to a square millimeter.

### 2.3. Meat emulsion samples

The meat emulsions used in this study were prepared in batches of 1 kg in a food processor (CombiMax 600, Braun, Germany). Thawed meat (480 g), potato starch (5 g), curing salt (NaCl with 0.6% nitrite) (17 g) and crushed ice (248 g) were comminuted at highest speed for 2 min. The temperature at this point was 1 °C in all batters. After addition of 250 g of either hand chopped cubes of lard or sunflower oil the batter was comminuted for 2 min. The temperature was measured (approx. 12 °C), and comminuting was continued for 1 min. End

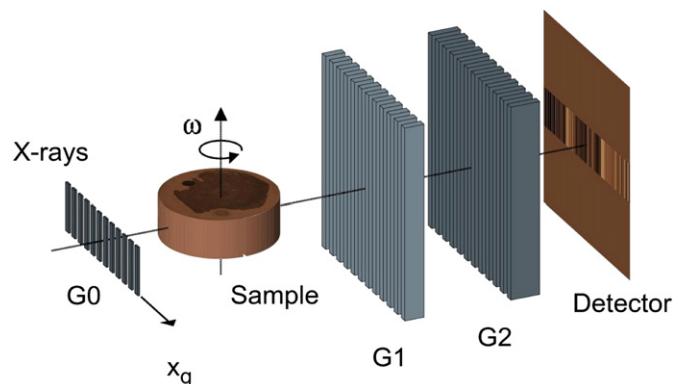


Fig. 2. A schematic of an X-ray tomography setup using a grating interferometer. Reprinted from Nielsen et al., 2012.

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