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Publication date:
2013

Citation for published version (APA):
Miklos, R., Lametsch, R., Nielsen, M. S., Lauridsen, T., & Einarsdottir, H. (2013). *Effect of fat type and heat treatment on the microstructure of meat emulsions*. Abstract from Inside Food, Leuven, Belgium.

Download date: 19. sep.. 2020

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Published in:
InsideFood Symposium

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Link to publication

Citation (APA):
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Effect of fat type and heat treatment on the microstructure of meat emulsions

Rikke Miklos[a], René Lametsch[a], Mikkel Schou Nielsen[b], Torsten Lauridsen[b], Hildur Einarsdottir[c]

a *) Department of Food Science, UCPH, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark*
b) Niels Bohr Institute, UCPH, Blegdamsvej 17, DK-2100 Copenhagen, Denmark
c) Dept. of Applied Mathematics and Computer Science, Technical University of Denmark, Matematiktorvet Bldg. 303B, DK-2800 Kgs. Lyngby, Denmark

ABSTRACT

In comminuted meat products the gel-forming abilities of the myofibrillar proteins are of major importance. In meat emulsions fat will be present in globules which are stabilized by a membrane coating made of salt-soluble proteins. These discontinuous fat particles act as fillers or co-polymers and stabilize the protein network. Differences in the physicochemical properties of saturated and unsaturated lipids affect the distribution of fat and thereby the functionality and quality of the final product. The objectives were to study the effects of lipid type and heat treatment on changes in microstructure of meat emulsions by use of a novel quantitative application of absorption- and phase-contrast tomography. The non-invasive technique offered the possibility to study the same sample in both raw and cooked condition. The samples were raw and heat treated meat emulsions (10% protein, 25% fat, 60% moisture) prepared with either pork fat or sunflower oil. The tomograms were obtained at a synchrotron facility using a grating interferometer which measured three different properties in the sample simultaneously: The attenuation length, the electron density and the diffusion length. Phase contrast imaging of the tomograms were used to analyse the impact of lipid type on spatial fat distribution, microstructure of the protein network and structural changes caused by heat treatment. The tomograms showed that the fat distribution in the meat emulsions depended on the physicochemical properties of the added fat. Use of vegetable oil resulted in homogeneous emulsions with smaller fat globules compared to the use of pork fat. This has previously been shown by the use of light micrographs. However, with the use of phase contrast imaging it was, from the same image, possible to resolve the protein phase to obtain information about the quality of the protein network and of the changes in microstructure caused by heat treatment. Further it was possible to compare the amounts of cooking loss from the emulsions. In conclusion phase contrast imaging with its high spectral resolution offers a unique possibility for studies of microstructure and is superior to histology since the information is obtained for the full volume.

1 Introduction

The most important functional characteristics in comminuted meat products are the gel-forming abilities of myofibrillar proteins. Traditionally animal fat is chopped into small globules of roughly 50 µ in diameter, which are stabilized by a membrane coating made of salt-soluble myofibrillar proteins. These discontinuous fat particles act as fillers or co-polymers and thereby stabilizing the myofibrillar network. Interactions of emulsified fat-globules with proteins influence the overall functionality and quality of the final product [Wu et al 2009]. Differences in the physicochemical properties of saturated and unsaturated lipids, i.e. emulsification properties and physical state, will affect the distribution of fat and influence on the protein-fat interaction in the product.

During the last decades X-ray computed tomography has generated interest as a valuable tool in non-destructive three-dimensional imaging of microstructure of various food products [Babin et al. 2006, Frisullo et al. 2009; Laverse et al. 2012] Recently a novel grating based X-ray phase-contrast tomographic method with increased contrast has been demonstrated [Bech et al. 2009, Weitkamp et al. 2005]. The objective of this study was to study the effects of lipid type and heat treatment on the microstructure of meat emulsions by the use of phase contrast tomography. Advanced data segmentation allowed quantitative parameters as percent objective volumes of the emulsion components, mean fat volumes of fat and water and cooking loss to be extracted from the data.

2 Material and Methods

2.1 Meat emulsions

The emulsions (aimed to contain: 10% protein, 25% fat, 60% moisture, 0.5% starch) were prepared in batches of 1 kilo in a food processor (CombiMax 600, Braun, Germany). Thawed minced pork meat (480 g), potato starch (5 g), curing salt (NaCl with 0.6% of 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 hand chopped cubes of lard or liquid sunflower oil the batter was comminuted for 2 min. The temperature was measured (approx. 12 °C) and comminuting was continued for 1 min. End temperature was 14 °C.

For tomography measurements a portion of meat emulsion was placed in an Eppendorf tube. To avoid air bubbles the samples were centrifuged at 5000g for 10 min, and had the lid closed under the surface of degassed PBS. After measurements of the raw samples the exact same samples were heat treated by placing the samples in a 200 mL glass of water heated in a microwave oven until the water reached the boiling point. The Eppendorf tube was left in the water bath for 15 minutes for the lard sample and 10 minutes for the sunflower oil sample. Both samples were then placed in a cold water bath, 10 minutes for the sunflower oil sample and 15 minutes for the lard sample.

2.2 X-ray tomography

Absorption, phase-contrast and dark-field CT scans of the meat emulsions were obtained by use of a grating interferometric set-up at the TOMCAT beamline at the Paul Scherrer Institute (PSI). The set-up is described in detail in [McDonald et al. 2009]. Measurements were made at 25 keV and the third Talbot fractional distance. The full volumes obtained - absorption, phase contrast and dark field - were 1720x1720x513 voxels, with an effective pixel size at sample of 7.4 μm.

2.3 Data segmentation

With multivariate analysis the data was segmented in two steps. First the data was modelled as a mixture of multivariate Gaussians with an expectation-maximization (EM) algorithm [Hastie et al. 2009]. Secondly, the data was segmented using an efficient approximate graph cut algorithm [Boykov et al. 2001]. These two steps ensure that both the multispectral and spatial context of the data is considered.

2 Results

Images of transverse cuts from each emulsion sample are presented in Fig. 1. The images are prepared from single slices of the reconstructed phase contrast data sets. The dark ring surrounding each emulsion is the Eppendorf tube used as sample container. In the raw sample prepared with lard (A) the fat phase is represented by large dark globules surrounded by a grey protein phase. When the lard emulsion was heated (B) the shape of the fat globules changed towards more spherical particles. The protein phase changed to a heterogeneous matrix with large light grey areas. Shrinkage of the emulsion structure as a consequence of protein denaturation is seen as decrease in the emulsion volume leading to formation of a slip between the emulsion and the container. The homogeneous grey phase that appears along the edge of the sample is a water phase which is assigned to the expected cooking loss of the emulsion. When the meat emulsions were prepared with sunflower oil (C) the structure consists of a homogenous protein phase with small black spots of fat and areas of a lighter grey protein phase. The continuous protein phase has a darker intensity compared to the lard samples due to inclusion of oil, which results in a lower intensity compared to pure protein. Heat treatment of the emulsion prepared with sunflower oil (D) did only result in small structural changes and limited shrinkage compared to the emulsion prepared with lard. The small white spots present in the two raw samples are insolubilized salt crystals.

The observed difference in microstructure can be further explored by comparison of the histograms provided for each sample (Fig 1, right side). The raw lard emulsion can be separated into a protein and a fat phase represented by two peaks in the histogram. After cooking the peak representing the protein phase broadens due to increased variation in the protein density. This variation may arise from shrinkage of some parts of the protein network resulting in increased density (light grey areas), whereas some of the protein will go into solution due to the presence of salt. Solubilised protein will result in lower density (grey areas). The density of the observed water phase is shifted from 0 to 1.8 indicating that water soluble proteins are included in this phase. The fat phase constituted by lard and minor amounts of intramuscular fat is shifted slightly towards a lower density.

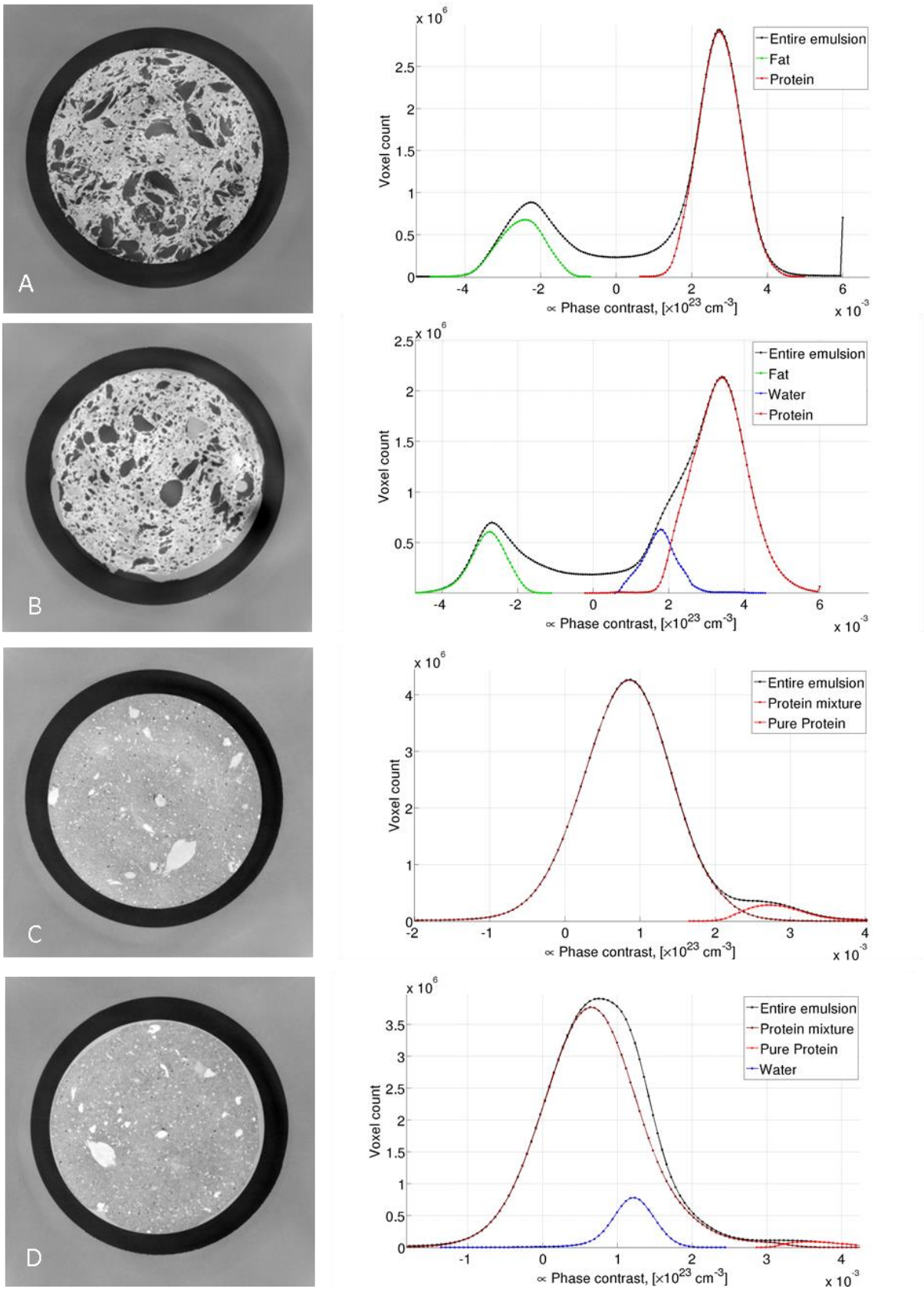

Fig 1. Left side: Reconstructed transverse slices of the emulsion samples. Right side: Histograms of the distribution of the emulsion components as segmented by multivariate contextual segmentation. A: Raw emulsion, Lard; B: Cooked emulsion, Lard; C: Raw emulsion, sunflower oil; D: Cooked emulsion sunflower oil.

From the histograms of the raw emulsions prepared with sunflower oil it is clear that only the insolubilized aggregated protein with high density can be separated from the rest of the emulsion. Due to resolution limitations it was impossible to separate the small fat globules from the homogeneous continuous protein phase. After cooking a peak representing a water phase is present in the histogram. It should be noted that this water phase has a slightly lower density compared to the water phase in the cooked lard emulsions.

From the data segmentation a quantitative analysis of the emulsion structures was performed. In Table 1 the percentage object volumes (POV) for each component are presented

Table 1 Per cent object volumes for the ingredients in the emulsion samples. The protein phases include both the sunflower oil and water which could not be separated due to resolution limitations.

Parameter	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
Protein (%)	73.9	59.4	98.1	89.9
Water (%)	-	15.6	-	9.6
Fat (%)	25.3	25.0	-	-
Salt (%)	0.8	-	1.7	-
Oil droplets (%)	-	-	0.2	0.5

Other quantitative parameters as mean fat volume, mean water volume, cooking loss and porosity are presented in Table 2. The cooking loss percentage gives the percentage of water surrounding the emulsion, which means that water entrapped inside the protein network is not included. The water populations within the sunflower oil emulsion have a smaller average volume, compared to lard emulsions, which contributes to the resulting homogeneity of the cooked emulsion.

Table 2 Quantitative parameters for the emulsion samples. Cooking loss gives the percentage of water separated from the emulsion, porosity is the pore volume divided by the total volume of the emulsion, anisotropy gives the degree of 3D symmetry in the emulsion structure, and the structure thickness is the average of the local thickness of the protein network.

Parameter	Lard raw	Lard cooked	Sunflower oil raw	Sunflower oil cooked
Mean fat volume (μm3)	$1.19 \cdot 10^6$	$9.80 \cdot 10^5$	-	-
Mean water volume (μm^3)	-	$1.68 \cdot 10^6$	-	$3.92 \cdot 10^5$
Cooking loss (%)	-	7.4	-	8.3
Porosity (%)	26.1	35.6	6.4	7.1
Anisotropy (-)	0.45	1	0.12	0.16
Structure thickness (μm)	47.36	33.3	100.64	68.08

3 Discussion

The tomograms showed that the fat distribution in the meat emulsions was influenced by the physicochemical properties of the added fat. Use of vegetable oil resulted in homogeneous emulsions with smaller fat globules compared to lard. Heat treatment caused more pronounced structural changes in the heterogeneous lard emulsions compared to the homogeneous oil emulsions. These well-known effects of the lipid phase and cooking on the gel stability have previously been shown by measurements of cooking loss and light micrographs [Youssef & Barbut, 2010, Wu et al. 2009]. However, the phase contrast tomograms made it possible to study the structural changes caused by heat treatment in the exact same sample. Besides data segmentation made it possible to extract and study structure related parameters quantitatively. The structural differences between the emulsions are visualised in Fig 1 and can be studied quantitatively by comparison of the parameters presented in Table 2. The parameters porosity and anisotropy increased markedly when the lard emulsion changed from raw to cooked, whereas the same parameters are at the same level for both the raw and the cooked sunflower emulsion. When the emulsions were prepared with lard it was possible to resolve variations within the protein network into different protein phases assigned to either aggregated (high density) or solubilised (lower density) (Fig 1). Quantification of these phases may be useful in future studies of the effects of addition of various functional components to meat emulsion products.

Further it was possible to quantify and compare the amounts of cooking loss from the emulsions. The cooking loss was determined to 7.4% and 8.3% for the lard emulsion and the sunflower emulsion respectively (Table 2). This relationship is in contrast to what was expected, as homogeneous gels generally are more stable compared to highly aggregated gels. The explanation can be found in the segmentation procedure as only water

pools expelled from the emulsion is defined as cooking loss in this case. However, when looking at the POV of the entire water populations (Table 1) the percentages found in the emulsions are 15.6% and 9.6% for lard and sunflower oil emulsions respectively. The higher degree of porosity of the lard emulsion prevents the water from expulsion and leads to formation of internal water pools, which are also visible at Fig 1 (B). It is further noted that the level of 15.6% of water separation is in good correspondence with previously findings for this emulsion type [Miklos et al. 2011].

The amount of protein in the cooking loss differed between the emulsion types. The density of the water phases in the heat treated emulsions (Fig 1) is lowest in the sunflower emulsion. This indicates that more protein was extracted to the water phase during heat treatment of the lard emulsion.

4 Conclusions

Phase contrast tomography offers the possibility to study structural changes of meat emulsions caused by cooking. The non-destructive characteristics of the method made it possible to study the exact same sample before and after heat treatment. In general the fat type had a major impact on the microstructure of the meat emulsions. When sunflower oil was used a homogeneous protein network containing small fat droplets was formed. When lard was used a heterogeneous protein structure containing fat globules in various sizes was formed. The high contrast in the images of lard emulsions made it possible to both visualise and quantify structural variation within the protein network. Due to resolution limitations it was impossible to resolve the small oil droplets from the protein phase when the emulsions were prepared with sunflower oil.

Acknowledgements

The authors gratefully acknowledge the experimental work by Rasmus Laurberg Hansen and Karin E. Ibsen on obtaining the sample data set. H.E., M.S.N. and R.M. acknowledge financial support through the NEXIM research project founded by the Danish Council for Strategic Research (contract no. 11-116226) within the Program Comission on Health, Food and Welfare.

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