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Ultra-structure of oil/water emulsions

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Addition of fish oil to industrial food products is of interest to both the food industry and consumers for reasons such as health benefits and added commercial value. Fish oil is rich in long chain omega-3 fatty acids, which contain a large number of double bonds. This causes the omega-3 fatty acids to be highly susceptible to oxidation. This feature limits the shelf-life of fish-oil enriched food products. Strategies for limiting oxidation and thus increasing the shelf-life of potential products are necessary for commercial production. One such strategy is to add the oil as an emulsion rather than as pure fish-oil. Studies so far have indicated that emulsification of the fish oil changes the oxidative stability of the product but whether emulsification is an advantage or not seems to depend on the food matrix to which the emulsion is added [1, 2]. It is therefore of interest to look at the pure emulsions to assess what determines the oxidation. It has been proposed that oxidation is, to some extent, dependent on the structure of the emulsion; including oil droplet size distributions and the thickness of the interface between oil and water. This interface can be stabilized by food grade emulsifiers such as proteins and phospholipids from milk.

The main objective of this study is to characterize fish oil/water emulsions focusing on oil droplet size distribution and characterization of the oil/water interface (thickness and organization). Because of the expected size of the interface layer, a few nm, electron microscopy appears to be the technique of choice to study these samples. We analysed several oil/water emulsions, stabilized by sodium caseinate as emulsifier, prepared in different ways: chemical fixation combined with/ room temperature embedding in resin, cryofixation combined with/ freeze substitution, and cryofixation combined with / cryo imaging (freeze-fracture cryo-SEM).

The high water content of these samples is the first problem to solve for the analysis of the samples. Therefore we developed agar pockets for encapsulation or used capillary tubes for conventional protocols (e.g. chemical fixation and room temperature embedding). Chemical fixation of these samples is challenging because of the need to minimize ultra-structural modifications of the samples especially during the dehydration step which can induce artefacts like collapsing and shrinkage. The chemical fixation appears to be a promising way to prepare the emulsions. Indeed, we obtained a first view of the emulsions and the organisation of the interface layer surrounding the oil droplets. Cryofixation is a preparation method, which allows observing the sample in a "close to native" state, and we could observe the oil/emulsifier/water interface more precisely. We observed that freeze fractured frozen samples observed in cryo-SEM (Figure 1) are really similar to the freeze substituted samples (Figure 2). With TEM we could even observe the network of protein surrounding the oil droplets (Figure 2).

With this work, we wish to demonstrate the need of combining different microscopic approaches to access the ultrastructure of the oil/water emulsions due to their complexity and instability.

1. N.S. Nielsen and C. Jacobsen. *Int J Food Sci Tech* 44 (2009) 1536-1546
2. M. B. Let, C. Jacobsen and A. S. Meyer. *J Agric Food Chem* 55 (2007) 7802-7809
3. The micrographs were recorded at Electron Microscopy Facility, Université de Lausanne and Center for Electron Nanoscopy, Technical University of Denmark.

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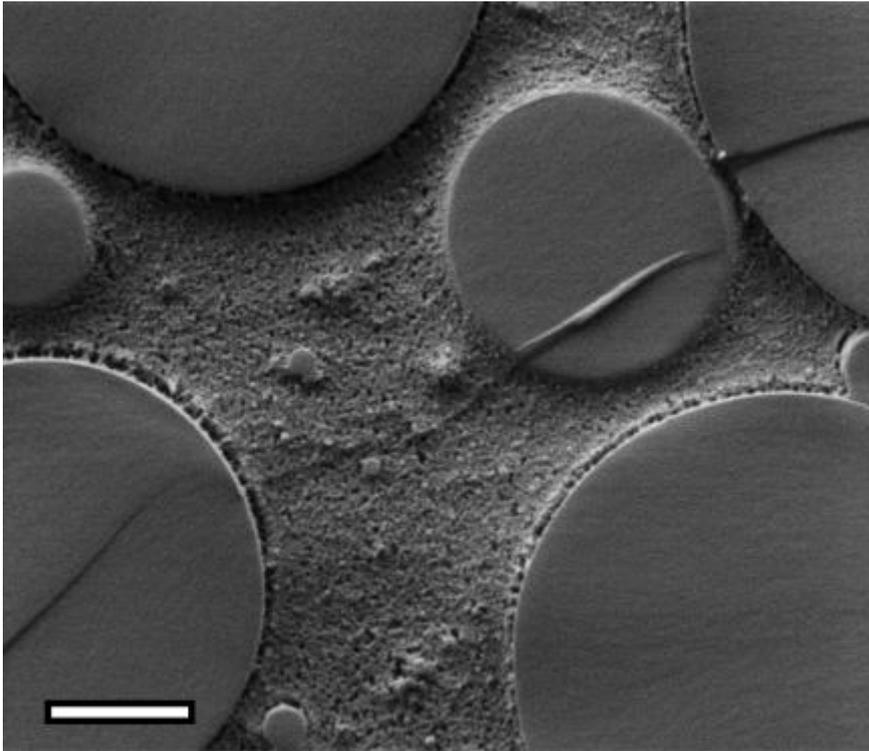


Figure 1. Emulsion containing 70% fish oil and 2.8% sodium caseinate. Freeze fracture cryo-SEM. Scalebar 2 μm .

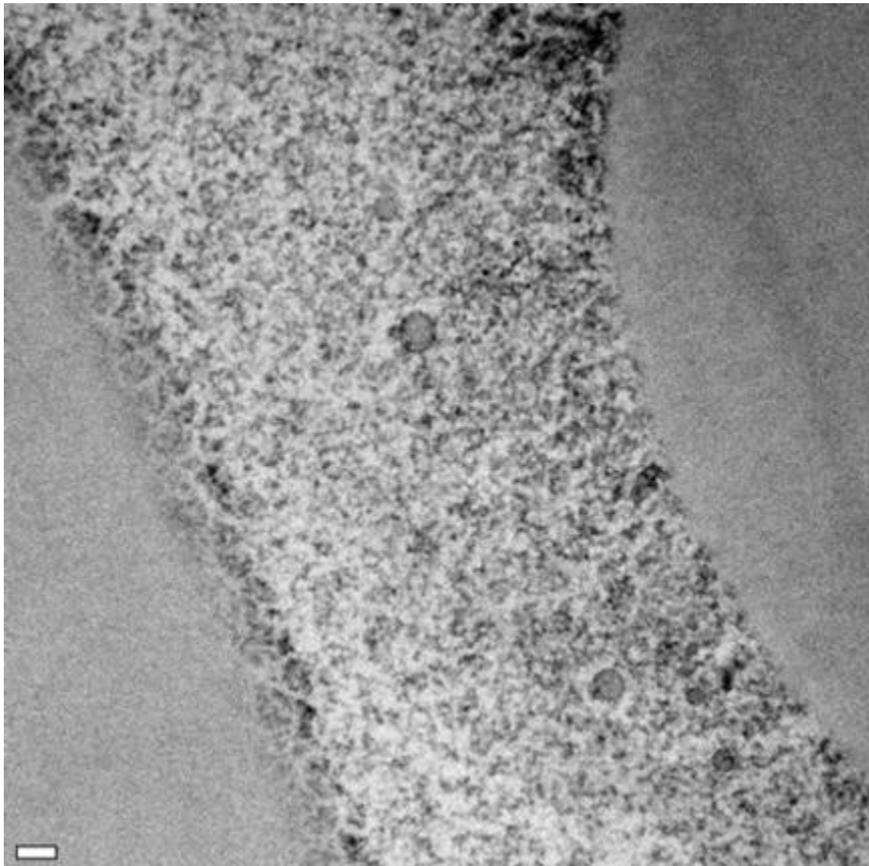


Figure 2. Emulsion containing 70% fish oil and 2.8% sodium caseinate. The micrograph depicts the edge of two oil droplets and the protein containing water phase between them. Freeze substitution, TEM. Scalebar 100 nm.