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Cryo-FIB SEM for Characterization of the Structure of Fish Oil Emulsions

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Introduction

Adding fish oil to food products to improve nutritional quality by the addition of omega-3 fatty acids is attractive both to the consumers and the food industry for reasons such as health benefits and added product value. The long chain omega-3 fatty acids contain a large number of double bonds which causes the fish oil to be susceptible to oxidation. The shelf lives of fish oil enriched products are thus limited by fast oxidation rates of the fish oil which causes development of off-flavours as well as degeneration of the beneficial health effects of the fish oil. At the present moment this is a barrier for their access to the market and it is necessary to develop techniques to protect the oil against oxidation. Emulsification of the oil has been put forward as a strategy for protection against oxidation, but whether that is beneficial seems to depend on the food matrix to which the oil is added (1,2); see figure 1. It is thus interesting to investigate the pure emulsions to gain knowledge about the oxidation without the effects of an external food matrix. It has been seen that some factors that influence the oxidation in pure emulsions are the type of emulsifier, the oil droplet size and the pH (3). This dependence has led to the belief that the oxidation is initiated at the interface between water and oil and that the thickness or composition of the interface can be controlled to ensure optimum stability of the emulsions.

Emulsifiers in this study and their effect on oxidative stability

The emulsifiers in this study were three milk phospholipid based emulsifiers with 75% phospholipid (MPL75) (2.8% w/w of emulsion); and sodium caseinate (NaCas) (2.8% w/w of emulsion).

Oxidation in emulsions with interface layers composed of either 2.8% NaCas or 2.8% MPL75 has been measured by Horn et al. (selected data from the study in (3)), see figure 2. These results indicate that 70% of oil emulsions emulsified with MPL75 are less stable against oxidation than both the pure oil and the emulsions stabilized by the protein based emulsifier NaCas.

Objective

We have previously investigated the impact of different emulsifiers on oxidation rates as well as investigated the microstructural differences between different types of emulsions (3). Cryo-SEM and freeze-fracture showed a difference in the appearance of the surface of the oil droplets in protein based and phospholipid based emulsions, if the latter had a high content of phospholipids (75% of the emulsifier), see figure 3 and 6, respectively. A high content of phospholipids in the emulsion did not cause a different surface appearance but also caused the fractures to follow the droplet surface; we could not get access to cross fractures of the oil droplets to see the interface layers in this sample. Additionally, the appearance of shells surrounding the oil droplets was suggested multiple layers of emulsifier, shown by black arrows in figure 6. Access to the interface layer could not be accomplished by embedding and sectioning because emulsion samples will collapse during the procedure (4). We have applied cryo-FIB-SEM to the samples in order to get access to the interface layer and to possibly verify multiple phospholipid layers around the oil droplets.

Method

Milling and imaging took place in a dual beam FEI Quanta FEG 3D. The emulsions were milled in copper rivets and plunged frozen in dry nitrogen (−210 °C) and transferred to a Quorum PFP2000T preparation system in vacuum. They were freeze-fractured and submitted to sublimation at −90 °C for (7.5−8 minutes) and sputter coated with Pt for (5−10 s). Milling and imaging was performed at −120 °C. Coarse milling was done at 20 kV and fine milling was done at 49 pA with beam energy 30 kV. The samples were imaged with ETD 5−10 kV and WD 10 mm.

Results

The samples were easy to mill, and milling resulted in almost smooth surfaces, even without cold deposition of Pt on the surface. We found that the samples could be imaged without sputtering extra Pt on the milled surface at various kV. This proved to be an advantage because it enabled us to see both the oil/water and secondary electron images on otherwise smooth surface, see figure 5. The oil droplets appear as dark circles and the ice appear bright. We were not able to detect visible multiple layers of milk phospholipids in the sample emulsified with MPL75, but due to the secondary electron contrast from the uncoated sample, we were able to locate some layers within the ice, see figure 7, right side. These might be excess emulsifier and/or eutectic ridges from the freezing process, same as are seen in the freeze fractured surfaces after sublimation.

Conclusions

This contradicts the view that the oil and water seems to be a promising way to distinguish between oil and water with secondary electrons in the smooth surfaces in the FIB-SEM. More work needs to be done to understand the contrast mechanisms from cryo-FIB-SEM of this type of sample.

References


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Figure 1. Oxidation in energy-bare enriched with fish oil, measured as amount of the unstable 1,4-2,4-hexadiynole 1-

Figure 2. Oxidation, measured as peroxide value, PC, traditionally defined as meq. per kg of oil.

Figure 3. Protein based emulsifier, 2.8% NaCas, freeze-fractured, etched and milled. Imaged uncoated at 3 kV. Image in right side is from the same area in higher magnification.

Figure 4. Contrast in secondary and back scattered electron image between (a) electron microprobe image (top), milled, shown in figure 5. The oil droplets appear as dark circles and the ice appear bright. We were not able to detect visible multiple layers of milk phospholipids in the sample emulsified with MPL75, but due to the secondary electron contrast from the uncoated sample, we were able to locate some layers within the ice, see figure 7, right side. These might be excess emulsifier and/or eutectic ridges from the freezing process, same as are seen in the freeze fractured surfaces after sublimation.

Figure 5. Protein based emulsified, 2.8% NaCas, freeze-fractured, etched and milled. Imaged uncoated at 3 kV. Image in right side is from the same area in higher magnification.

Figure 6. Milk phospholipid based emulsifier, 2.8% MPL75, freeze-fractured, etched and milled. Imaged uncoated at 30 kV. Image in right side is from the same area at the bottom side of the oil droplet in higher magnification.

Figure 7. Milk phospholipid based emulsifier, 2.8% MPL75, freeze-fractured, etched and milled. Imaged uncoated at 30 kV. Image in right side is from the same area at the bottom side of the oil droplet in higher magnification.

Figure 8. Milk phospholipid based emulsifier, 2.8% MPL75, freeze-fractured, etched and milled. Imaged uncoated at 30 kV. Image in right side is from the same area at the bottom side of the oil droplet in higher magnification.