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# The choice of homogenisation equipment affects lipid oxidation in emulsions

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ABSTRACT

Milk proteins are often used by the food industry because of their good emulsifying properties. In addition, they can also provide oxidative stability to foods. However, different milk proteins or protein components have been shown to differ in their antioxidative properties, and their localisation in emulsions has been shown to be affected by the emulsification conditions. The objective of this study was to investigate the influence of homogenisation equipment (microfluidizer vs. two-stage valve homogeniser) on lipid oxidation in 10% fish oil-in-water emulsions prepared with two different milk proteins. Emulsions were prepared at pH 7 with similar droplet sizes. Results showed that the oxidative stability of emulsions prepared with sodium caseinate was not influenced by the type of homogeniser used. In contrast, the type of homogenisation equipment significantly influenced lipid oxidation when whey protein was used as emulsifier, with the microfluidizer resulting in lower levels of oxidation.



## 1. Introduction

Lipid oxidation in emulsions is expected to be initiated at the interface between oil and water. The emulsifier used to build the interfacial layer might therefore be crucial for the resulting oxidative stability. The type of emulsifier determines the structure and thickness of the interfacial layer (Hunt & Dalgleish, 1994a) and emulsifiers can furthermore have different antioxidative properties (Faraji, McClements, & Decker, 2004; Haahr & Jacobsen, 2008). Moreover, the oil droplet size has in some cases been shown to influence lipid oxidation (Jacobsen et al., 2000; Kargar, Spyropoulos, & Norton, 2011; Sun & Gunasekaran, 2009). This was explained by the fact that smaller droplets have a larger contact area between prooxidative transition metal ions in the water phase and lipid hydroperoxides present at the oil–water interface. Oil droplet size is known to be influenced by different factors such as the type and concentration of the emulsifier used (Horn et al., 2011; Qian & McClements, 2011), the emulsification equipment and the homogenisation conditions (Dalgleish, Tosh, & West, 1996; Let, Jacobsen, Sorensen, & Meyer, 2007; Robin, Remillard, & Paquin, 1993).

The bovine milk proteins, caseins and whey proteins, are commonly used as emulsifiers by the food industry because of their good emulsifying and physically stabilising properties. Whereas casein consists of mainly four different components ($\alpha_{s1}$-, $\alpha_{s2}$-, β- and κ-casein), whey protein has two major protein components (α-lactalbumin and β-lactoglobulin). During emulsification they easily adsorb at the surface of the oil droplets with the more hydrophobic amino acid regions projecting into the water phase, and the less hydrophobic regions facing the oil phase (Krog, 2004). However, the surface hydrophobicities of individual protein components have been suggested to be of less importance for the adsorption rate and emulsifying abilities than the flexibility of the conformational structure (Shimizu, Kamiya, & Yamauchi, 1981). The caseins are very flexible in nature, due to the lack of higher structures (Creamer, 2003). Hence, in low concentration casein is expected to stretch over the oil droplet surface, whereas in high concentration the casein molecules are expected to form more compact structures at the interfacial layer and they therefore protrude longer into the water phase. This will result in the formation of interfacial layers ranging from 5 to 10 nm (Fang & Dalgleish, 1993). In contrast, the flexibility of whey proteins depend on a reduction of their tertiary and quartenary structures, as influenced by e.g. emulsion formation, protein concentration, pH or heating (Fang & Dalgleish, 1997, 1998; Lee, Lefèvre, Subirade, & Paquin, 2007).

In addition to the structure of the proteins, also the amino acid compositions of the two types of proteins give them different antioxidative properties. Caseins, but not whey proteins, contain several phosphorylated serine residues that have been suggested

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to possess metal chelating effects (Diaz, Dunn, McClements, & Decker, 2003; Hekmat & Mcmahon, 1998). In contrast whey proteins have more sulfhydryl groups that are suggested to scavenge free radicals (Elias, McClements, & Decker, 2005; Tong, Sasaki, McClements, & Decker, 2000).

If the pH of the emulsion is above or below the isoelectric point of the milk proteins, the interfacial layer will be electrically charged, which can prevent droplet flocculation by electrostatic repulsion. Moreover, the surface charge can influence lipid oxidation, with positive charge giving rise to reduced oxidation due to repulsion of the cationic transition metal ions (Hu, McClements, & Decker, 2003a).

In the industry two kinds of high pressure homogenisation systems are widely used: The valve homogeniser and the microfluidizer (Schultz, Wagner, Urban, & Ulrich, 2004). Both devices are mainly used for secondary homogenisation, meaning the disruption of larger droplets into smaller ones in an already prepared premix. The valve homogeniser has a pump that pulls the emulsion into a chamber on its backstroke, and then forces it through a narrow valve at the end of the chamber on its forward stroke. In the valve, intense disruptive forces will cause the larger droplets to break down into smaller ones. The microfluidizer also has a pump that drives the emulsion through to a chamber with a very small passage, in which the droplets are forced to collide, and thereby reduce in size (Schultz et al., 2004). A previous study on milk has shown that homogenisation in a microfluidizer resulted in a different location of the individual protein components at the interfacial layer or in the aqueous phase, when compared to homogenisation in a valve homogeniser (Dalgleish et al., 1996). Furthermore, structural differences in the milk proteins present at the interface were observed between milk homogenised in the two equipments.

Based on these previous studies, it was hypothesised that emulsification by different homogenisation equipments would influence the structure of the proteins at the interfacial layer and their partitioning into the aqueous phase, and that these differences would affect lipid oxidation. Furthermore, it was hypothesised that whey protein emulsions would oxidise more than casein emulsions when iron was added, due to the metal chelating effect of phosphorylated serine residues in casein. Thus, the aim of this study was to compare lipid oxidation in 10% fish oil-in-water emulsions prepared on a microfluidizer or a two stage high pressure valve homogeniser. Emulsions were made with either 1% sodium caseinate or whey protein isolate as emulsifier, and lipid oxidation was catalysed by iron addition. Emulsions were characterised, visually inspected using cryogenic transmission electron microscopy (cryo-TEM) and followed by oxidation stability studies.

## 2. Materials and methods

### 2.1. Materials

Commercial cod liver oil was provided by Maritex A/S, subsidiary of TINE, BA (Sortland, Norway) and stored at −40 °C until use. The fish oil was described by its fatty acid composition, content of tocopherols and peroxide value (PV). The fatty acid composition was determined by preparation of methyl esters (AOCS, 1998b) that was in turn analysed by gas chromatography (AOCS, 1998a). The content of the major fatty acids was (in area% of total fatty acids) as follows: 14:0 3.0, 16:0 8.9, 16:1($n$−7) 8.2, 18:1($n$−9) 16.0, 18:1($n$−7) 5.2, 18:4($n$−3) 2.5, 20:1($n$−9) 11.6, 20:5($n$−3) 9.3, 22:1($n$−11) 6.1 and 22:6($n$−3) 11.6. The levels of tocopherols were determined by HPLC (AOCS, 1998c). Tocopherol contents were 207 ± 16 μg α-tocopherol/g oil and 100 ± 1 μg γ-tocopherol/g oil. The initial PV of the fish oil was <0.1 meq peroxides/kg oil, as determined by the method described in Section 2.4.1. Sodium caseinate (Miprodan® 30) and whey protein isolate (Lacprodan® DI-9224) were kindly donated by Arla Foods Ingredients amba (Viby J, Denmark). Data sheets from Arla reported a protein content of 93.5% in sodium caseinate and 92% in whey protein isolate. All other chemicals and solvents used were of analytical grade.

### 2.2. Preparation of emulsions and sampling

Emulsions were prepared with 10.0% (w/w) fish oil, 89.0% (w/w) 10 mM sodium acetate imidazole buffer (pH 7.0) and either 1.0% (w/w) sodium caseinate or 1.0% (w/w) whey protein isolate. Emulsions were produced in batches of 600 g. Prior to emulsification the protein was dissolved in the buffer overnight, and fish oil was then added slowly during mixing at 16,000 rpm (Ystral mixer, Ballrechten-Dottingen, Germany). The fish oil was added during the first minute of mixing, and the total mixing time was 3 min. Secondary homogenisation was done either on a microfluidizer (M110L Microfluidics, Newton, MA, USA) equipped with a ceramic interaction chamber (CIXC, F20Y, internal dimension 75 μm), or a two-valve high-pressure homogeniser (Panda 2K, GEA, Niro Soavi, Parma, Italy). The experimental design with sample code names, applied pressures and number of passes in the homogeniser is listed in Table 1. The differences in homogenisation pressures and number of passes were used to obtain equal droplet size distributions for emulsions prepared at the two different emulsification equipments by the same emulsifier. Emulsions were added 100 μM $FeSO_4$ to accelerate lipid oxidation and 0.05% (w/w) sodium azide to prevent microbial growth. Emulsions were stored in 100 ml Bluecap bottles at room temperature (19–20 °C) in the dark for 14 days. Samples were taken at day 0, 4, 7, 10 and 14. Measurements of viscosity and pH were done at day 0, droplet sizes were measured at day 1 and 14. Two emulsions of each type were prepared.

### 2.3. Characterisation of the emulsions

#### 2.3.1. Droplet size, viscosity and pH

Droplets were measured by laser diffraction in a Mastersizer 2000 (Malvern Instruments, Ltd., Worcestershire, UK). Emulsion was diluted in recirculating water (3000 rpm), until it reached an obscuration of 12–14%. The refractive indices of sunflower oil (1.469) and water (1.330) were used as particle and dispersant, respectively. Measurements were made in duplicate. The initial emulsion viscosities were determined using a Brookfield viscometer Model RV DV II (Brookfield Engineering Labs. Inc.,

**Table 1**
Experimental design.

| Emulsion | Emulsifier | Homogeniser | Pressure | No of passes[a] |
|---|---|---|---|---|
| CAS_M | Sodium caseinate | Microfluidizer | 10.000 psi (69 MPa) | 3 |
| CAS_H | Sodium caseinate | Valve homogeniser | 80/8 MPa | 4 |
| WPI_M | Whey protein isolate | Microfluidizer | 10.000 psi (69 MPa) | 3 |
| WPI_H | Whey protein isolate | Valve homogeniser | 50/5 MPa | 3 |

[a] Number of passes run on the homogeniser.