FACULTY OF **BIOSCIENCE ENGINEERING**

Interaction between whey proteins and lipids as a result of light-induced oxidation

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Introduction

Dairy products are a suitable group for **ω-3 polyunsaturated fatty acid (PUFA) fortification**, but are very susceptible to photo-oxidation due to the presence of the **photosensitizer riboflavin**. Unlike ordinary triplet oxygen oxidation, light-induced oxidation is characterized by a quick formation of highly reactive singlet oxygen and free radicals, even at low storage temperatures. Besides vitamins, dairy fats and proteins are the primary targets for light-induced oxidation (Figure 1).

In this study, the interaction between whey proteins and lipids with a different degree of unsaturation (olive, soybean, fish and algae oil) was investigated during photo-oxidation of oil-in-water emulsions.

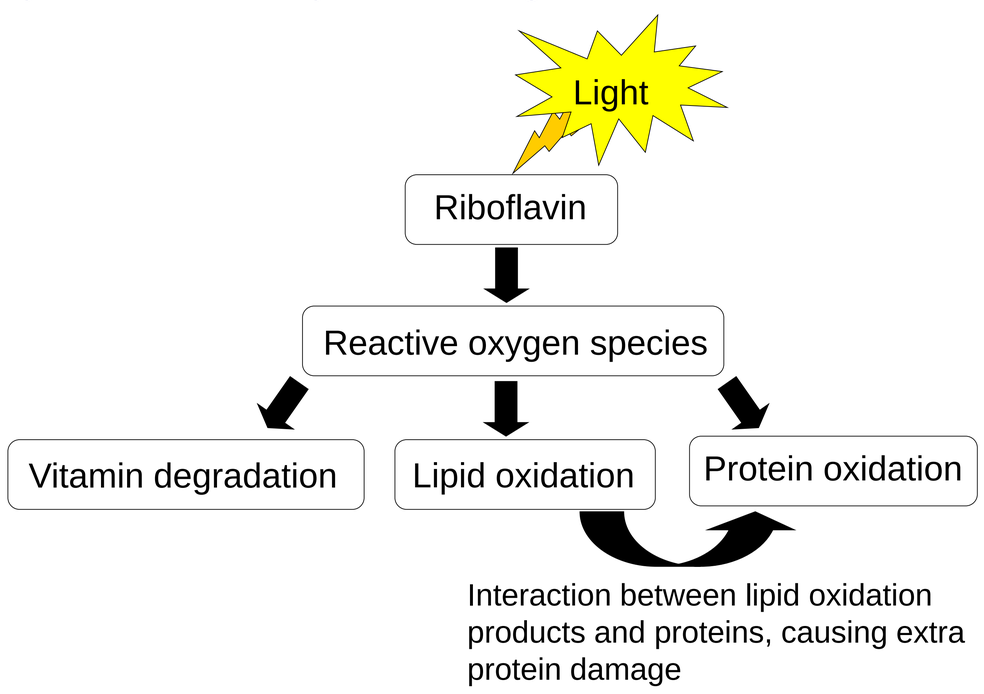
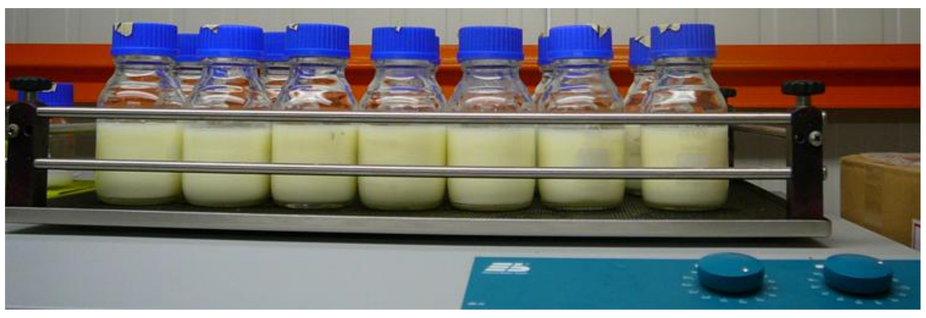


Figure 1. Overview of photo-oxidation process and its consequences

Materials and methods



Oil-in-water emulsions were prepared in phosphate buffer by homogenizing 10mg/mL olive, soybean, fish or algae oil and 6mg/mL whey protein isolate, in the presence of 2 µg/mL riboflavin. The emulsions were illuminated at 1500 lux (4°C).

Protein oxidation was assessed by the amount protein-bound carbonyl groups (DNPH) and by measurement of the amino acid profile as well as gelelectrophoresis.

Results and discussion

Riboflavin degraded exponentially upon light exposure, irrespective from the degree of oil unsaturation, with a complete degradation after 13 illumination days. In the lipid phase, the PUFA fraction was preferentially degraded, creating extra reactive oxygen and carbonyl species.

More protein oxidation was measured in the emulsions richer in unsaturated lipids, as evaluated by the protein-bound carbonyl groups (Figure 2) and tryptophan content (Figure 3). The essential amino acids tryptophan, lysine, tyrosine and methionine were most sensitive to photo-oxidation. Protein oxidation continued even after complete degradation of the photosensitizer.

The oxidized lipids chemically interacted with the whey protein, stimulating the formation of protein carbonyls (Figure 2) and protein cross-links. Accordingly, protein aggregates of high molecular mass were formed upon prolonged illumination (Figure 4).

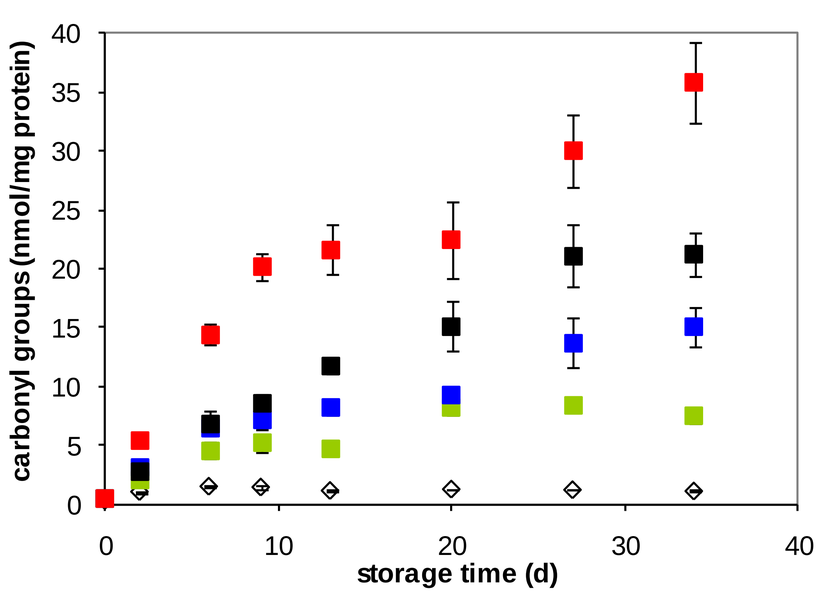


Figure 2. Protein carbonyl formation in light-exposed **olive**, **soybean**, **fish** and **algae** oil emulsions as compared to emulsions stored in the dark ( )

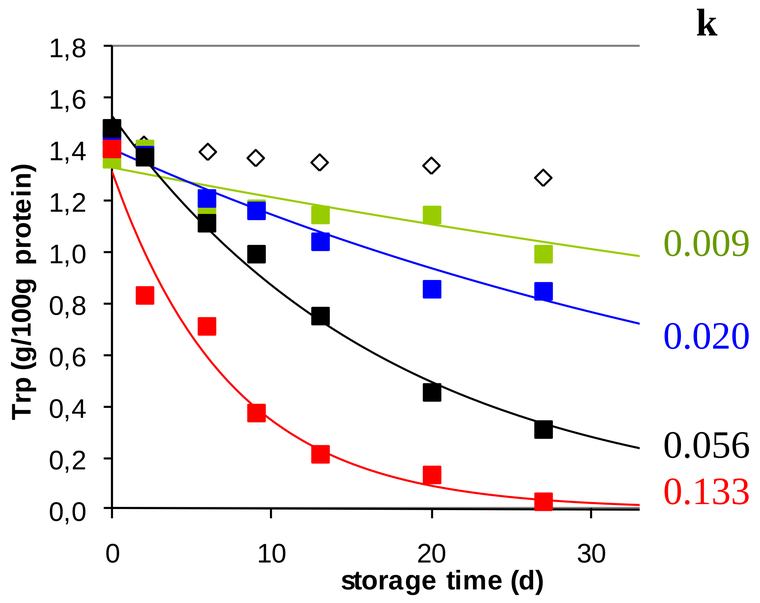


Figure 3. Exponential tryptophan degradation in light-exposed **olive**, **soybean**, **fish** and **algae** oil emulsions as compared to emulsions stored in the dark ( ) (k = reaction rate constant)

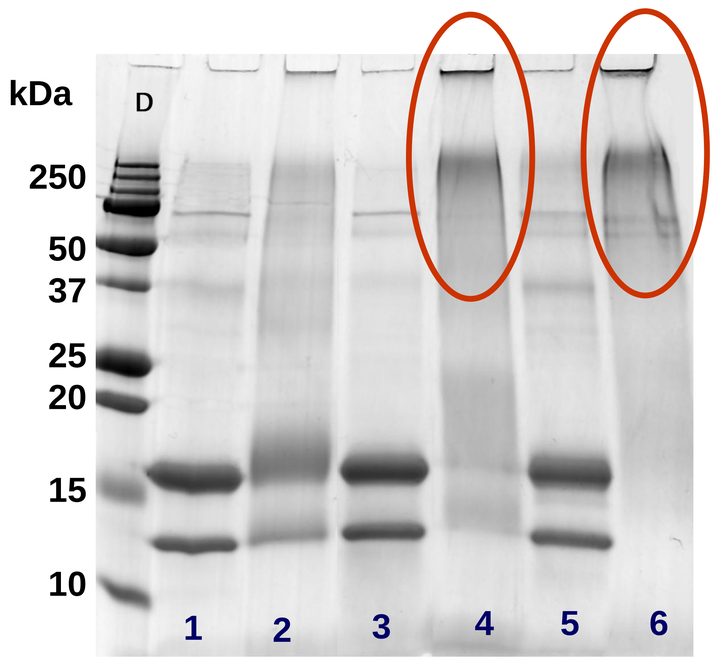


Figure 4. SDS-PAGE of protein extracts of algae oil emulsions [1] day 0; [2] day 6 light; [3] day 13 dark; [4] day 13 light; [5] day 34 dark; [6] day 34 light

Conclusions

- Presence of PUFA intensified the photo-oxidation process of the whey proteins.
- The oxidation process continued even after complete degradation of the photosensitizer.
- Photo-oxidation had a major impact on the nutritional value: essential amino acids and PUFA were preferentially degraded.
- A good protection of PUFA-enriched dairy products, through packaging material, micro-encapsulation of the PUFA fraction or use of antioxidants is essential in order to safeguard the nutritional benefits of these foodstuffs.

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