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**Studies on the antioxidant activity of milk proteins
in model oil-in-water emulsions**



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Abstract

The present study was aimed at extending our knowledge of the antioxidative properties of the milk protein products, whey protein isolate (WPI) and sodium caseinate (NaCas), in oil-in-water (O/W) emulsions rich in polyunsaturated fatty acids (PUFAs). In particular, the objective was to contribute to our understanding of the compositional and processing factors that influence the oxidative stability of protein-stabilised O/W emulsions. Linoleic acid (approximately 60 %) was used as the lipid for the oil phase (10.6 %). The emulsion samples were usually incubated at 50 °C to accelerate lipid oxidation. Lipid oxidation indicators were lipid hydroperoxides and headspace hexanal, determined by solid phase microextraction (SPME) combined with gas chromatography (GC).

WPI- or NaCas-stabilised emulsions were prepared using a wide range of protein concentrations (0.5, 1.0, 2.0, 3.0, 4.0, 7.0 or 10.0 %) at two droplet sizes ($d_{32} = 0.31$ and $0.65 \mu\text{m}$). In general, higher lipid oxidation levels were found for the larger droplet size. Increasing protein concentration led to a decrease in the lipid oxidation rate. The greatest decrease in lipid hydroperoxide levels (values after 4 h) occurred at up to 4.0 % protein concentration. The greatest decrease in hexanal levels (values after 24 h) occurred at up to 4.0 % protein concentration in WPI emulsions ($0.31 \mu\text{m}$). The hexanal levels were more independent of the protein concentration in the other emulsion types. The hexanal level decreased at protein concentrations > 4.0 % in NaCas emulsions (0.31 and $0.65 \mu\text{m}$) and at protein concentrations > 7.0 % in WPI emulsions ($0.65 \mu\text{m}$). The difference between lipid hydroperoxide generation in emulsions with small and large droplet sizes decreased with increasing protein concentration. This effect was more pronounced in NaCas emulsions. In general, NaCas was a better inhibitor of lipid oxidation than WPI, but WPI appeared to be the better antioxidant at some droplet size/protein concentration combinations.

The protein in the continuous phase, i.e. the unadsorbed protein, played an important role in lipid oxidation. In principal, the lipid hydroperoxide and hexanal levels showed the same development over the continuous phase protein concentration as over the

protein concentration in WPI and NaCas emulsions ($d_{32} = 0.31 \mu\text{m}$). A low NaCas level in the continuous phase already led to a relatively low hexanal level, whereas a higher WPI level was required. When NaCas solution was added to a WPI emulsion or WPI solution was added to a NaCas emulsion, a synergistic antioxidative effect was observed.

The high molecular weight fractions (molecular weight ≥ 12000 – 14000) of WPI and NaCas contained pro-oxidative metal ions that contributed to lipid oxidation in the emulsions. An enrichment of NaCas emulsions with the low molecular weight fraction of NaCas (with a molecular weight ≤ 12000 – 14000) notably inhibited lipid oxidation. An enrichment of WPI emulsions with the low molecular weight fraction of WPI (with a molecular weight ≤ 12000 – 14000) also seemed to inhibit lipid oxidation, but the effect was not significant. The protein solutions were enriched with these fractions before emulsion preparation.

Pure WPI solution or mixed WPI/NaCas (1:1, weight/weight) solution with 1.12 or 2.24 % protein concentration was heated at $84 \text{ }^\circ\text{C}$ for up to 40 min, cooled and then used to prepare emulsions. Lipid oxidation was generally not affected by the heat treatment or the degree of whey protein denaturation. However, at the lower WPI concentration, more hexanal was produced for the longer heating times (20, 30 and 40 min) and this appeared to be connected with the physical instability of the emulsions. Greater oxidative stability was found at the higher protein concentration and when the proteins were mixed, pointing to a possible synergistic antioxidative effect of WPI and NaCas.

The addition of the free radical source 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) greatly increased the oxygen uptake and the generation of lipid hydroperoxides in the emulsions. The oxidative stability increased with increasing protein concentration (1.0, 4.0 and 7.0 %). NaCas had a greater antioxidative effect than WPI. The inhibition of oxygen uptake appeared to be largely influenced by the free-radical-scavenging activity of the system, determined by the protein type and the protein concentration, as the radicals were produced linearly over time and oxygen was consumed linearly over time. It can therefore be concluded that free-radical-scavenging activity represents a major antioxidative mechanism of the milk proteins.

Oxygen was consumed much faster in emulsions than in protein solutions when the same level of AAPH was incorporated. In a WPI (1.0 % protein) emulsion, much lower levels of protein hydroperoxides than of lipid hydroperoxides developed. This pointed to a much greater reactivity of linoleic acid than of the milk proteins with oxygen. In contrast, the exposure of WPI to oxidising linoleic acid in an emulsion (1.0 % protein) or to AAPH in aqueous solution led to oxidative damage of the whey proteins, indicated by the loss of amino acids. The loss of specific amino acids was different for proteins in the continuous phase or cream phase of an emulsion or in WPI solution.

The present study confirms the antioxidative potential of WPI and NaCas and gives new insights into their functionality as oxidative stabilisers in O/W emulsions.

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Table of contents

| | |
|---|-----------|
| Abstract | i |
| Acknowledgements | v |
| Table of contents | ix |
| Chapter 1: Introduction | 1 |
| Chapter 2: Literature review | 5 |
| 2.1 Lipids | 5 |
| 2.1.1 Fatty acids in food material | 5 |
| 2.1.2 Fatty acids and human health | 6 |
| 2.2 Oxidation of unsaturated fatty acids | 8 |
| 2.3 Measuring lipid oxidation | 10 |
| 2.3.1 Oxygen consumption | 10 |
| 2.3.2 Lipid hydroperoxides | 10 |
| 2.3.3 Conjugated diene hydroperoxides | 11 |
| 2.3.4 2-Thiobarbituric acid (TBA) value | 11 |
| 2.3.5 Carbonyl compounds | 12 |
| 2.3.6 Gas chromatographic (GC) analysis | 13 |
| 2.4 Milk proteins | 14 |
| 2.4.1 Caseins | 14 |
| 2.4.2 Whey proteins | 15 |
| 2.5 Oil-in-water (O/W) emulsions | 17 |
| 2.6 Lipid oxidation in O/W emulsions | 21 |
| 2.6.1 Role of pro-oxidative metal ions, chelators, the pH and the surface charge | 21 |
| 2.6.2 Primary antioxidants | 25 |
| 2.6.3 Oxygen | 26 |
| 2.6.4 The droplet interface as a physical barrier | 27 |
| 2.6.5 Viscosity | 28 |
| 2.6.6 Oil phase concentration | 28 |
| 2.6.7 Droplet size | 29 |
| 2.6.8 Retention of volatiles | 32 |

| | | |
|-------------------|--|-----------|
| 2.7 | Antioxidative influence of milk proteins in O/W emulsions and similar systems | 33 |
| 2.7.1 | General findings | 33 |
| 2.7.2 | Influence of continuous phase protein on oxidative stability | 35 |
| 2.7.3 | Influence of heat treatment of milk proteins on oxidative stability | 36 |
| 2.7.4 | Influence of high and low molecular weight protein fractions, protein hydrolysates and peptides on oxidative stability | 39 |
| 2.8 | Antioxidative mechanisms of milk proteins, milk protein hydrolysates and peptides | 41 |
| 2.8.1 | Hydrophobicity | 42 |
| 2.8.2 | Metal binding | 43 |
| 2.8.3 | Free-radical-scavenging ability | 50 |
| Chapter 3: | Materials and methods | 59 |
| 3.1 | General materials | 59 |
| 3.1.1 | Emulsion lipid | 59 |
| 3.1.2 | Proteins | 59 |
| 3.1.3 | Chemicals for lipid hydroperoxide and hexanal determination | 59 |
| 3.2 | General methods | 60 |
| 3.2.1 | Determination of the iron and copper content of the protein powders | 60 |
| 3.2.2 | Preparation of protein solutions | 60 |
| 3.2.3 | Preparation of emulsions | 60 |
| 3.2.4 | Acceleration of the lipid oxidation rate by emulsion storage at elevated temperature | 61 |
| 3.2.5 | Measurement of the droplet size | 62 |
| 3.2.6 | Measurement of the pH value | 62 |
| 3.2.7 | Lipid hydroperoxide determination | 62 |
| 3.2.8 | Hexanal determination | 63 |
| 3.2.9 | Spectrophotometric measurements | 64 |

| | | |
|---|---|------------|
| 3.2.10 | Statistical analysis | 64 |
| 3.3 | Materials and methods in Chapter 4 | 65 |
| 3.3.1 | Methods: Chapter 4.1 | 65 |
| 3.3.2 | Methods: Chapter 4.2 | 65 |
| 3.3.3 | Methods: Chapter 4.3 | 66 |
| 3.4 | Materials and methods in Chapter 5 | 67 |
| 3.4.1 | Materials: Chapter 5 | 67 |
| 3.4.2 | Methods: Chapter 5.1 | 68 |
| 3.4.3 | Methods: Chapter 5.2 | 68 |
| 3.5 | Materials and methods in Chapter 6 | 69 |
| 3.5.1 | Materials: Chapter 6 | 69 |
| 3.5.2 | Methods: Chapter 6 | 70 |
| 3.6 | Materials and methods in Chapter 7 | 73 |
| 3.6.1 | Materials: Chapter 7 | 73 |
| 3.6.2 | Methods: Chapter 7 | 74 |
| 3.6.3 | Methods: Chapter 7.1 | 74 |
| 3.6.4 | Methods: Chapter 7.2 | 76 |
| Chapter 4: Factors affecting lipid oxidation in milk-protein-based O/W emulsions | | 79 |
| 4.1 | Effect of droplet size on lipid oxidation in NaCas-based linoleic acid emulsions | 80 |
| 4.2 | Effect of droplet size, protein type and protein concentration on lipid oxidation in linoleic acid emulsions | 86 |
| 4.3 | Role of unadsorbed protein in lipid oxidation | 97 |
| 4.4 | Addition of milk protein solutions to milk-protein-based emulsions and the influence on lipid oxidation | 104 |
| 4.5 | General discussion | 109 |
| Chapter 5: Influence of low molecular weight (LMW) compounds on lipid oxidation | | 119 |
| 5.1 | Influence of the removal of LMW compounds of WPI and NaCas by dialysis and the addition of metal ion chelators on lipid oxidation | 119 |

| | | |
|-------------------|---|------------|
| 5.2 | Effects of LMW fractions on oxidative stability | 134 |
| Chapter 6: | Effect of heat treatment of milk protein solutions prior to emulsification on the oxidative stability of O/W emulsions | 141 |
| Chapter 7: | Effect of free radicals on lipid and protein oxidation | 159 |
| 7.1 | Effects of free radicals on oxygen consumption and lipid hydroperoxide production in emulsions | 159 |
| 7.2 | Effects of free radicals on protein oxidation | 169 |
| 7.3 | General discussion | 186 |
| Chapter 8: | Concluding discussion and recommendations | 197 |
| 8.1 | Concluding remarks | 197 |
| 8.2 | Recommendations for future work | 208 |
| Appendix | | 211 |
| References | | 219 |
