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


A thesis presented in partial fulfilment of the requirements
for the degree of

Doctor of Philosophy
in
Food Technology

by

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2009

# 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 µm). 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 µm). 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 µm) and at protein concentrations > 7.0 % in WPI emulsions (0.65 µm). 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 µm). 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 ˚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.

# Acknowledgements

I am sincerely grateful to my chief supervisor, Professor Harjinder Singh, for his guidance, encouragement and generous help throughout the entire project. I highly appreciate his inputs and expertise. I especially thank him for preventing me from following the wrong tracks during the practical work, and for his support and guidance in the preparation of this thesis.

I am sincerely grateful to my co-supervisor, Dr Derek R Haisman. I would like to thank him for believing in my suitability for this project and bringing me to the Riddet Institute and Massey University. Also, I would like to thank him for his constant guidance with expertise, his support and encouragement throughout the practical work and during the preparation of the thesis.

I would like to thank Dr Aiqian Ye for his valuable inputs and expertise in questions about oil-in-water emulsions and his help in preparing publication material based on the findings of this project.

I wish to thank the Riddet Institute for the scholarship that enabled me to do this project.

I would like to thank Ms Leiza Turnbull, Ms Michelle Tamehana, Ms Fliss Jackson, Mr Garry Radford, Mr Steve Glasgow, Dr Kelvin Goh, Dr Lara Matia-Merino and Mr Warwick Johnson (Institute of Food, Nutrition and Human Health, Massey University), Ms Fliss Stibbards, Dr Peter Zhu, Ms Janiene Gilliland, Ms Maggie Zhou, Mr Shane Rutherfurd and Mr Chris Hall (Riddet Institute, Massey University) for their cooperation, help or assistance in experimental work or their advice. Thank you to Mr Matt Levin and Mr Peter Jeffery for their services in information technology (Institute of Food, Nutrition and Human Health, Massey University).

Thank you to Professor Eric Ainscough for his advice and the demonstrations in ESR spectroscopy, to Mr Grant Platt for his customised technical equipment used in one of the experiments (both Institute of Fundamental Sciences, Massey University), to Mr

Rob Hunter for his assistance in gel electrophoresis (Fonterra Co-operative Group Limited), to Dr Caryl Lockhart for her advice in statistical analyses (EpiCentre, Massey University) and to Dr Yacine Hemar for his inspiring contribution to the project (The MacDiarmid Institute for Advanced Materials and Nanotechnology).

A special thank-you I direct to my fellow post-graduates from the Seed Tech Hut and colleagues, to my friends and flatmates and other people I shared time with in the past years, for their company, their support, a word that cheered me up when it was tough, their prayers, for extending my knowledge in life science ☺ or insights into another culture of this world: Dr Noorzaid Muhamad, Dr Mary Eastham, Claire Hastings, Kathleen and Rick Field, Tim Coogan, Alex Kordichev, Nandu Goswami, Aniket Abhyankar, Dr Amelie Deglaire, Anwesha Sarkar, Catootjie Nalle, Dr Mischa Walton, Oni Yuliarti, Dr Supat Chaiyakul, Dr Ahmed Mohammed Rida Amerah**,** Thanuja Herath, Dr Janina Kühn, Prabhu Balan, Emmanuelle Riou, Hilary McKinnon, Visaka Anantawat, Jiahong Su, Sylvia Chung, Carolina Saavedra, Natalie Keller, Xuemei Tang, Cheryl Nkhasi, Dr Simon Loveday, Amit and Nimi Taneja, Dr Reza Mozafari, Dr Inge Merts, Dr Ajay Awati, Bonny Zhan, Liwei (Daisy) Bai, Anne Göppert, Taka Fihaki, Wiremu and Trieste Te Awe Awe, Ron Sanders, Daniel Chua, Francis Keete, Reuben Wallis, Hone Davis, Dr Caryl Lockhart, Benjamin Trembarth, Steven Evans, Birgit Schauer, Dr Simone Titus, Manfred Sutter, James and Alice Curtis, Ariunaa and Amra Mendtsoo, Dr Clyton Moyo, Bernard Phiri, Carlos Hug de Belmont, Dione Joseph, Dominic Tehan, Stephen Smith, Rachel Anderson, Emma Dodson, Penelope Byrnes, Christina Rock, Agatha Chiu, Aberry, James Valtorta, Lucy Ellis, Lynda Bottrill, Sonya House, Trudy Leslie, Albertha Lavi, Jane Dravitski, Averil Wills, Alex Ogidi, Deogratius Mohanga, Fuminori Watanabe, Kunsik Park, Kieran Udy, Ellen Schoner, Fr Marcus Francis, Fr Brian Walsh, Jonny Boon and Mary-Grace Williams, Professor Janina Mazierska, David Mullin, Kui and Helen Chen, Giovanna, Tatiana Shirokova, Sonja Koch, Karin Kohler, Stefanie Bachmann, Dominik Durner, Monique Laurie, Philipp Schenkel, Morgane Blanc, Florence, Christoph Rau, Michaela Eckstein, Kaustubh Savant, Andre Bruns, Helga Kocurek, Jozsef-Sebastian and Katrin Pap, Paul and Dianne Stock, Matthias and Victoria Herzog, Joanne Oliver, Lucia Kwa and all members of the Magnificat Community, Cross Creek, and Freedom Church, Te Rangimarie Marae, Rangiotu.

I would like to thank my family, especially my Mum, my Dad and my brothers, who were far away but still there for me when I needed them.

Finally, I want to thank God for holding me in his hands, although he does not always give me a hand. I am grateful to come to the end of this PhD journey.

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