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Massey University

**Structure and properties of liposomes prepared
from milk phospholipids**

**A thesis presented in partial fulfilment of the requirements for the degree
of Doctor of Philosophy in Food Technology**

By

Abby Kerrin Thompson

Riddet Centre and Institute of Food, Nutrition and Human Health

Massey University, Palmerston North, New Zealand

2005

Abstract

The isolation of milk fat globule membrane (MFGM) material from buttermilk on a commercial scale has provided a new ingredient rich in phospholipids and sphingolipids. The aim of this project was to explore the possibility of producing liposomes from MFGM-derived phospholipid material (Phospholac) and to compare the properties of these liposomes with those produced from commercial soy phospholipid fractions (SigP3644 and Ultralec). The technique used for liposome production was to be suitable for use in the food industry.

All three phospholipid fractions were primarily composed of phosphatidyl choline and phosphatidyl ethanolamine, but the dairy-derived Phospholac also contained approximately a third sphingomyelin. It also had a more highly saturated fatty acid profile, and contained a significantly higher proportion of protein than the soy-derived fractions.

The phospholipid fractions were dispersed in an aqueous system and cycled through a Microfluidizer[®] (a high-pressure homogeniser) to successfully produce liposomes. These were then characterised using a wide range of techniques.

The hydrodynamic diameter of the liposomes, measured using Photon Correlation Spectroscopy, ranged from an average of ~95 nm for the Phospholac dispersion to ~80 nm for the SigP3644 and Ultralec samples. All three dispersions had a very wide particle size distribution. Electron microscopy showed that all three dispersions appeared to be primarily unilamellar, but there was a small percentage of multilamellar and multivesicular liposomes. The unilamellar nature of the dispersions was further supported by the small-angle X-ray diffraction images and ³¹P-NMR results.

The SigP3644 dispersion had a much higher permeability than either the Phospholac or Ultralec sample, with minimal difference between the Phospholac and Ultralec samples at either 20 or 40 °C. Differential scanning calorimetry (DSC) found that SigP3644 and Ultralec had phase transition temperatures below 0 °C, while Phospholac dispersions showed a very broad transition with a centre between 28 and 30 °C. However, these differences did not appear to relate to the membrane permeability at its phase transition temperature. The Phospholac and Ultralec bilayers were approximately 20% thicker than SigP3644 membranes, with no significant change in thickness between 20 and 40 °C.

The liposomes produced from the Phospholac fraction showed considerably improved stability under a variety of environmental conditions than those produced from soy phospholipids. The Phospholac dispersions were able to withstand more severe processing treatments, were stable for longer periods at higher storage temperatures, and were less affected by changes in pH and in ionic concentration. It is thought that these differences are due to the high sphingomyelin concentration and more saturated fatty-acid profile of the dairy-derived fraction.

There were noticeable differences in entrapment characteristics of the fractions. It was found that the entrapment efficiency of hydrophobic compounds was directly proportional to the solubility of the compound in the solvent phase used for dispersion. Hydrophilic entrapment was also investigated, but the rapid diffusion of the small hydrophilic molecules through the liposome membrane prevented quantification of the entrapment efficiency. To produce liposome dispersions suitable for the encapsulation of hydrophilic material, further work must be completed to reduce the membrane permeability.

Differences in the properties of the liposome dispersions appear to be related to the composition differences between the phospholipid fractions, and it may be possible to exploit the unique composition of the MFGM phospholipid material in the delivery of bioactives in functional foods.

Acknowledgements

I have been very fortunate with the support and assistance that I have received throughout the course of this research project. Many people have freely and graciously helped with my work and impacted positively on my life during the last 4 years, and I take this opportunity to convey my sincere thanks to them.

It was not until I was well into my project that I appreciated the wisdom of the saying: 'chase your supervisor, not your topic'. I am very grateful to my chief supervisor, Professor Harjinder Singh, for his thoughtful commentary, practical advice and faith in my ability. Dr Derek Haisman was usually my first port of call when I needed to discuss a new idea or problem. He quickly and graciously read all of my first drafts and listened kindly and sympathetically whenever I needed reassurance. I am also grateful to Assoc Professor David Harding, for his softly-spoken advice, and for returning my manuscripts before anyone else with an apology for taking so long; and to Dr Palatasa (Tasa) Havea, for gently manoeuvring me into the Dairy Industry in the first place.

I would like to thank all of the people who provided technical skills and support, without which I would have been floundering. From Fonterra Innovation, Drs Skelte Anema and Edwin Lowe for all their advice and assistance with the Zetasizer and AFFF, and Robyn Hirst for performing the Thin-section TEM. Dr Thomas Rades and his team from the National School of Pharmacy, University of Otago, for helping with the characterisation of the liposomes, particularly through providing the Cryogenic SEM images. From the Institute of Fundamental Science, Professor Geoff Jameson for performing the small-angle x-ray diffraction measurements and Dr Pat Edwards for his assistance with the use of NMR to measure liposome lamellarity and entrapped volume. I also would like to thank Dr Jason Hindmarsh (Riddet Centre) for explaining to me how NMR may be used to measure membrane permeability; Dr Alisdair Noble (Institute of Information Sciences and Technology) for assisting with statistical analyses; Dr Colin Downes (Industrial Research Ltd) for providing the Differential Scanning Calorimetry results; Ashley Duncan (University of Otago) for the GC analysis of fatty acids and Dr Phil Pearce (Institute of Food, Nutrition and Human Health) for his assistance in measuring phospholipid concentrations.

Thanks also to Xiang Zhu (Peter) and Michelle Tamehana (both of the Riddet Centre) and Warwick Johnson (Institute of Food, Nutrition and Human Health) not only for their technical help, but more importantly for their friendship and advice on the world at large.

Thank you to my friends and flatmates, who have been tolerant when I snapped at them after yet another mechanical failure, tried to understand when I headed to bed early again because I was just too tired, and were willing to talk for hours about anything other than my thesis. Hopefully by the time any of you read this, you will never again have to tentatively enquire whether we are still not talking about the PhD!

I am extremely lucky to belong to a family who consider education a natural and important part of life. I have often turned to my parents for guidance and reassurance during my research and the preparation of this thesis, and am extremely grateful for their sympathetic, practical advice. They were always there to remind me that if it was easy, everyone would have a PhD; but never wavered in their total confidence that I would (eventually) have one myself.

I also wish to thank two of my best friends: Adrian, who helped me get through the highs and lows of the first years of my thesis and who has continued to have unconditional faith in me; and Timothy, for his patience, encouragement and for ensuring I never forget that there is more to life than work. My friendship with both of them has added to my enjoyment and understanding of life and of people, and I hope we will continue to challenge and support each other in the years ahead.

Finally, I'd like to share two quotes which have kept me company throughout this part of my life. They have helped me keep everything in perspective, especially during the preparation of this thesis, emphasising that things do not necessarily have to work in order to be worthwhile, and reminding me that with determination, perseverance and sheer hard work almost anything can be achieved.

"Make everything as simple as possible, but not simpler."

Albert Einstein

"I have not failed. I've just found 10,000 ways that won't work."

Thomas Alva Edison

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