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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

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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 matieral (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 ^{31}P-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.

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"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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