J. Dairy Sci. 89:410–419

Preparation of Liposomes from Milk Fat Globule Membrane Phospholipids Using a Microfluidizer

A. K. Thompson and H. Singh[1]
Riddet Centre, Massey University, Private Bag 11 222, Palmerston North, New Zealand

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. An MFGM-derived phospholipid fraction was used to produce liposomes via a high-pressure homogenizer (Microfluidizer). This technique does not require the use of solvents or detergents, and is suitable for use in the food industry. The liposome dispersion had an average hydrodynamic diameter of 95 nm, with a broad particle-size distribution. Increasing the number of passes through the Microfluidizer, increasing the pressure, or reducing the phospholipid concentration all resulted in a smaller average liposome diameter. Changing these variables did not have a significant effect on the polydispersity of the dispersion. Electron microscopy showed that the dispersions formed had a range of structures, including unilamellar, multilamellar, and multivesicular liposomes. The composition of the MFGM phospholipid material is different from that of the phospholipids usually used for liposome production in the pharmaceutical and cosmetic industries. The MFGM-derived fraction comprises approximately 25% sphingomyelin, and the fatty acids are primarily saturated and monounsaturated. These differences are likely to affect the properties of the liposomes produced from the phospholipid material, and it may be possible to exploit the unique composition of the MFGM phospholipid fraction in the delivery of bioactive ingredients in functional foods.
Key words: milk fat globule membrane, liposome, encapsulation, Microfluidizer

INTRODUCTION

Whole milk contains approximately 0.035% phospholipid, of which approximately 35% is in the milk serum, and the remaining 65% is in the milk fat globule membrane (**MFGM**). The MFGM phospholipids are primarily phosphatidyl choline (**PC**), phosphatidyl ethanolamine (**PE**), and sphingomyelin (**SM**), with small amounts of phosphatidyl serine and phosphatidyl inositol (**PI**).

Phospholipids have been shown to have a number of health benefits, including liver protection (Koopman et al., 1985) and memory improvement (Crook et al., 1991, 1992). Sphingolipids are required for cellular signaling, and have been shown to be involved in the control of cell proliferation, apoptosis, inflammation, and cancer (Huwiler et al., 2000). Sphingomyelin also inhibits intestinal absorption of cholesterol and fat in rats, with milk SM being more effective than egg SM (Peel, 1999). Sphingolipids are traditionally extracted from bovine brain, and are not only very expensive but also unsuitable for use in foods for vegetarians. Issues relating to bovine spongiform encephalopathy and Creutzfeldt-Jakob disease make it undesirable to use material extracted from bovine brain in food systems. The relatively high concentrations of sphingolipids in dairy phospholipid could avoid many of the negative issues surrounding many of the other sources of phospholipids and sphingolipids.

The identification of these biological functions of phospholipids, in particular sphingolipids, has led to increasing interest in techniques for isolating phospholipid fractions from waste dairy streams, such as buttermilk. These techniques range from traditional methods using solvent extraction to emerging technologies such as microfiltration and supercritical fluid extraction (Astaire et al., 2003; Corredig et al., 2003). Currently, the Fonterra Cooperative Group Ltd. (New Zealand) is the only company known to extract and purify MFGM phospholipid fractions from buttermilk commercially. In addition to their biological functions, MFGM phospholipids have been to shown to have good emulsification properties (Corredig and Dalgleish, 1998; Roesch et al., 2004), and have been used for the production of emulsions for drug delivery (Sato et al., 1994; Yuasa et al., 1994).

In the pharmaceutical and cosmetic industries, highly purified phospholipids extracted from soy oil or egg yolk are used to produce liposomes. Liposomes are spherical structures consisting of one or more phospho-

Received August 30, 2005.
Accepted October 4, 2005.
[1]Corresponding author: h.singh@massey.ac.nz

lipid bilayers enclosing an aqueous core (Zeisig and Cämmerer, 2001). They may be used for the entrapment and controlled release of drugs or nutraceuticals, as model membranes or cells, and for specialist applications such as gene delivery (Lasic, 1998).

There are many potential applications for liposomes in the food industry, ranging from the protection of sensitive ingredients to increasing the efficacy of food additives. However, the high cost of the purified soy and egg phospholipids, combined with problems in finding a production method suitable for use in the food industry, has limited the use of liposomes in foods. To our knowledge, MFGM-derived phospholipids have never been used in making liposomes. The high levels of sphingolipids in MFGM phospholipids may provide nutritional benefit for the consumer, as well as improved liposome functionality.

There are several methods that may be used to produce liposomes, and a number of excellent reviews have been published that provide preparation details of the more common production techniques (Watwe and Bellare, 1995; Betageri and Kulkarni, 1999; Frezard, 1999). The standard preparation procedure is via the rotary evaporation of a chloroform solution of phospholipid, cholesterol, and other hydrophobic compounds to produce a thin phospholipid film. Addition of water and hydrophilic compounds causes bilayer sheets of the lipid to separate from the bulk and form liposomes (Picon et al., 1994). Jackson and Lee (1991) stated that the large-scale production of liposomes was limited by poor encapsulation efficiencies, the lack of a continuous production process, and the use of organic solvents. They concluded that the solution to this problem might be the use of a microfluidization technique. The Microfluidizer is a high-pressure homogenizer that can rapidly produce a large volume of liposomes in a continuous and reproducible manner (Chen et al., 2001), without use of sonication, detergents, solvents, or alcohols. The liposome population produced appears to be relatively stable, without rapid aggregation or fusion (Kim and Baianu, 1991). In microfluidization, the phospholipid and the material to be entrapped are dispersed in a liquid phase. This may be water, an aqueous buffer solution, or a solvent, depending on the solubilities of the components. The solution is pressurized in continuous flow, and split into 2 streams that are then forced together at high velocity (>500 m/s). The resulting release of kinetic energy provides the required activation energy to break up the large phospholipid bilayer sheets into smaller fragments (Kim and Baianu, 1991). To minimize surface energy, the ends wrap around, forming bilayer vesicles known as liposomes.

The objective of the research presented in this paper was to study the formation of liposomes using a microfluidization technique. A phospholipid-rich fraction isolated on an industrial scale from MFGM was used to prepare liposomes.

MATERIALS AND METHODS

A phospholipid-rich fraction derived from MFGM (Phospholac 600) was provided by the Fonterra Cooperative Group Ltd. (New Zealand). It contained approximately 83% lipid, 6.2% lactose, 11.5% ash, and 2.6% moisture. All chemicals and other materials used were of analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO).

Fatty Acid Profile of Phospholipid Fraction

The lipid was extracted from the phospholipid fraction using chloroform and methanol in a ratio of 1:2 (by volume). Fatty acids were methylated by acid-catalyzed transesterification at 80°C for 12 h in a sealed tube. The fatty acid methyl esters were separated using a BPX-70 capillary column, 100 m × 0.22 mm i.d., 0.25-μm film (SGE, Melbourne, Australia). The gas chromatographic system consisted of a model 6890 gas chromatograph equipped with an autosampler (HP7673) and Chem Station integration (all Hewlett Packard, Avondale, PA). The column oven was held at an initial temperature of 165°C for 52 min, and then increased at a rate of 5°C/min to a final temperature of 210°C for 59 min (total run time: 120 min). Both the injector port and the flame-ionization detector port were at 250°C. The carrier gas flow (helium) was maintained at 1.0 mL/min (linear gas velocity: 20 cm/s) throughout the temperature program with an inlet split ratio of 30:1. Fatty acid peaks were identified by retention time matching with authentic standards, including a composite standard made from commercially available methyl esters (NuChek Prep, Elysian, MN; Sigma, St. Louis, MO).

Analysis of Phospholipid Head Group

^{31}Phosphorus-nuclear magnetic resonance (^{31}P-NMR) analysis was conducted on the MFGM phospholipid material in its original powder state. The analysis was performed by Spectral Service (Köln, Germany) using a Bruker AC-P 300-MHz NMR spectrometer (Diehl, 2001, 2002).

Preparation of Liposome Dispersions

A 10% lipid dispersion was made in imidazole buffer (20 m*M* imidazole, 50 m*M* sodium chloride, and 0.02% sodium azide in Milli-Q water, adjusted to pH 7, with