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Composition, Thermotropic Properties, and Oxidative Stability of Freeze-Dried and Spray-Dried Milk Fat Globule Membrane Isolated from Cheese Whey

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ABSTRACT: The milk fat globule membrane (MFGM) was isolated from cheese whey using a recently developed novel method. The cheese-derived MFGM contained about 17−19% lipids and 65−70% protein on a dry weight basis. About 50% of the lipids in MFGM were phospholipids. Compositional analysis of the cheese whey-derived MFGM showed that it is a rich source of phosphatidylserine, sphingomyelin, and bioactive proteins CD36, butyrophilin, xanthine oxidase, and mucin 1. Utilization of MFGM in foods as a source of nutraceutical lipids depends on its oxidative stability. In this context, the impact of drying methods, namely, freeze-drying versus spray-drying, on the storage stability of MFGM was studied. Freeze-dried (FD) and spray-dried (SD) MFGM samples were morphologically very different when examined by light microscope: The thermotropic phase transition temperature (T_m) of lipids in the FD-MFGM was 37.8 °C, and it was 48 °C in SD-MFGM. This 10 °C difference in T_m indicated that the drying method altered the thermodynamic state of phospholipids in MFGM. At all storage temperatures studied, the zero-order rate constant of lipid oxidation, as measured by hexanal production, was 1−2 orders of magnitude greater in the spray-dried than in the freeze-dried MFGM. The results clearly indicated that the choice of drying method affects morphological characteristics, the T_m and oxidative stability of phospholipids in MFGM.

KEYWORDS: milk fat globule membrane, oxidative stability, bioactive lipids, bioactive milk proteins, thermotropic phase behavior, phase transition temperature

INTRODUCTION

Milk is considered to be a whole food designed by nature to provide macro- and micronutrients as well as bioactive compounds to stimulate the growth of vital tissues and to protect the newborn against bacterial and viral infections. In recent years, the milk fat globule membrane (MFGM) has been receiving major attention as it is considered to be a source of several bioactive lipids and proteins vital for the survival and growth of the neonate.[1−3] For instance, the MFGM protein lactadherin is implicated in the modulation of neovascularization;[4] butyrophilin, which constitutes 40% of total MFGM proteins, is believed to suppress multiple sclerosis;[5] and xanthine oxidase and other glycoproteins have been shown to be antimicrobial agents.[6,7] In addition, several studies suggest that MFGM-derived phospholipids and sphingolipids have beneficial effects on the prevention of colon cancer, maturation of the gut, and myelination of the developing central nervous system in neonates.[3,8] Phosphatidylserine, which is present in significant amount in MFGM, is believed to be involved in brain development and cognitive function in infants.[9,10] The beneficial effects of proteins and lipids of MFGM in human health have been reviewed recently.[1]

Apart from its beneficial health effects, MFGM isolate has been shown to possess desirable emulsifying properties.[11,12] Thus, MFGM isolates from bovine milk can be used both as a standalone bioactive/nutraceutical ingredient and as an emulsifying agent in food products, including infant and geriatric foods. In addition, currently, soybean is the main source of food-grade lecithin available to the food industry. However, an alternative source of lecithin to cater to the needs of a segment of the population that is allergic to soybean products would be desirable. In this respect, bovine MFGM isolates can be used as the starting material for the production of dairy lecithin.

Utilization of MFGM in foods as a source of bioactive lipids and proteins depends on its oxidative stability. A typical preparation of MFGM isolate contains about 25% phospholipids and 70% proteins.[13] Because phospholipids in MFGM are associated with proteins in a trilayer membrane environment, their oxidative stability in the membrane environment might be very different from that in an isolated state. In addition, the drying method used in the preparation of MFGM isolates might affect the microenvironment of the lipids in the membrane and thereby their oxidative stability. Changes in the microenvironment of membrane lipids can be detected by studying the thermotropic properties of the membrane lipids. To the best of our knowledge there is no such information available in the literature. In the present study, we isolated the MFGM from cheese whey using a novel method[14] and studied its thermotropic properties and oxidative stability at three different storage temperatures.

MATERIALS AND METHODS

Materials. Clarified and pasteurized mozzarella cheese whey was obtained from a local cheese plant in Wisconsin. The pH of the cheese was about 6.4. Ethyl pentanoate (99%) and hexanal (97%) were from Sigma-Aldrich (St. Louis, MO).

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Isolation of MFGM from Cheese Whey. The MFGM was isolated from cheese whey using the method described elsewhere.[14] Briefly, cheese whey was first ultrafiltered 5-fold in a dairy pilot plant using a spiral wound 10 kDa molecular weight cutoff polysulfone membrane. Subsequently, the retentate was diafiltered 3-fold in a continuous mode using water. The final conductivity of the diafiltered retentate was about 500 μS cm^{-1}. The pH of the retentate was adjusted to 4.20 and incubated for 30 min at 35 °C. The retentate was then centrifuged at 10000 rpm in a desludger. A portion of the desludged solids, which is the MFGM material, was resuspended in water and freeze-dried. The remaining portion of the desludged solids was spray-dried in a pilot-scale spray-dryer.

Proximate Analysis of MFGM. The fat content of MFGM was determined according to the Mojonnier method.[15] The fatty acid composition of MFGM lipids was performed by Rtech Laboratories (St. Paul, MN) based on Association of Official Analytical Chemists Method 996.06 (AOAC 18th ed., modified 2001). The protein content was determined according to the Kjeldahl method using 6.38 as the nitrogen factor.

Differential Scanning Calorimetry. The thermal melting behavior of freeze-dried (FD) and spray-dried (SD) MFGM isolates was studied using a differential scanning calorimeter (DSC) (Micro DSC model VII, Setaram, Caluire, France) as follows: The MFGM suspension (10% w/w) in 10 mM phosphate buffer (pH 7.0) was accurately weighed into preweighed DSC vessels (Hastelloy C276) and closed tightly with the stopper. A matching DSC vessel containing the same amount of buffer was used as reference. Samples were heated from 5 to 115 °C at a constant heating rate of 1 °C/min. After each scan, the sample was cooled to 5 °C and rescanned from 5 to 115 °C. The melting temperature (T_m) and the enthalpy change (ΔH) were determined from the thermogram using Setsoft software (version 1.4) supplied by the DSC manufacturer. The DSC was calibrated using cyclohexane, phenyl ether, and *o*-terphenyl standards recommended by the DSC manufacturer. DSC analysis was carried out at least in triplicate for all samples.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). The protein profile of MFGM proteins was examined by SDS-PAGE according to Laemmli.[16] A 4−15% acrylamide gradient ready gel (Bio-Rad Laboratories, Inc., Hercules, CA) was used. The MFGM samples (10% w/w stock solution in 10 mM phosphate buffer, pH 7.0) were diluted to 1.0 mg/mL (protein basis) with the sample buffer (4% SDS, 20% glycerol, 10% β-mercaptoethanol, 0.125 M Tris, pH 6.8) and then heated in boiling water for 5 min. An aliquot of 15 μL per lane was loaded onto the gel. Electrophoresis was performed with a Mini-PROTEAN 3 cell (Bio-Rad Laboratories). A cocktail protein standard consisting of proteins in the molecular weight range of 10−250 kDa (Bio-Rad Laboratories) was also run along with the samples. The gels were stained in EZBlue gel staining reagent (Sigma-Aldrich). Scanning of the wet gels was done on the GS-710 calibrated imaging densitometer (Bio-Rad Laboratories) by using Quantity One software provided by the manufacturer.

^{31}P NMR. The ^{31}P NMR analysis was performed on the freeze-dried MFGM sample. Approximately 1 g of MFGM was dissolved in 100 mL of a detergent solution, and the ^{31}P analysis was performed by Avanti Polar Lipids, Inc. (Alabaster, AL) using a Bruker AVANCE III 400 MHz NMR spectrometer. The ^{31}P response was calibrated with a dioleoylphosphatidylcholine standard.

Light Microscopy. The morphological characteristics of the freeze-dried and spray-dried MFGM samples were analyzed using a Nikon microscope equipped with high-speed live display color camera head DS-Vi1 (Nikon Precision Inc., Belmont, CA). The MFGM sample powder (~0.1 g) was dispersed in 0.5 mL of mineral oil and votexed for 1 min. Two drops of the MFGM sample dispersions were placed onto a plain microscope slide and covered with a glass coverslip (Fisher Scientific, Pittsburgh, PA). The samples were observed under the microscope with 40× magnification, and the images were taken using the digital camera.

Determination of Oxidative Stability. To obtain uniform particle sizes, the freeze-dried and spray-dried MFGM isolates were passed through a 28 mesh sieve beforehand. The sieved samples were placed in an environmental chamber (model 34790-30, Cole Parmer, Vernon Hills, IL), which was prefluxed with nitrogen gas and pre-equilibrated with saturated LiCl solution (water activity = 0.249 at 25 °C) and incubated for 1 week. After equilibrium had been reached (water activity = 0.256), 0.2 g portions of the samples were exactly weighed inside the environmental chamber (glove compartment) and transferred to glass vials (17 × 60 mm, Fisher Scientific, Pittsburgh, PA) capped with Teflon-lined screw caps. The vials were stored in dark incubation chambers maintained at 25, 35, and 45 °C. Vials were withdrawn (in triplicate) at various time intervals for analysis of aroma volatiles.

Analysis of Headspace Volatiles by Gas Chromatography−Mass Spectrometry (GC-MS). Aroma volatiles in MFGM samples were analyzed by headspace GC-MS analysis using the solid-phase microextraction (SPME) technique as described elsewhere.[17] In a typical experiment, 2 mL of deionized water was added into each vial containing 0.2 g of MFGM. The vials were recapped, and the dispersions (10%) were stirred for 3 min at room temperature using a magnetic stirrer. Then, to each vial was added 25 μL of a stock solution (100 ppm) of ethyl pentanoate as internal standard, and the vial was then stoppered with a Teflon septum and incubated in a Multi-Blok heater (Barnstead Lab-line, Cardinal Health, Dublin, OH) at 37 °C. A SPME needle was inserted into the vial, and the SPME fiber (50/30 μm DVB/Carboxen/PDMS, Supelco, Bellefonte, PA) was exposed to headspace for 40 min at 37 °C. Volatiles were desorbed from the SPME fiber at 220 °C for 5 min in the injection inlet of a Hewlett-Packard HP6890 series gas chromatograph (Hewlett-Packard, Palo Alto, CA) and separated on a Restek RTX-5MS capillary column (30 m × 0.25 mm, 0.5 μm thickness) (Restek Inc., Bellefonte, PA) using helium as carrier gas at a constant flow rate of 0.9 mL/min. For GC analysis, the GC oven temperature was maintained at 35 °C for 8 min, increased from 35 to 120 °C at the rate of 5 °C/min, held at 120 °C for 1 min, then ramped from 120 to 250 °C at the rate of 11 °C/min, and held at 250 °C for 3 min. Volatiles eluting from the column were routed to an Agilent 5973 mass spectrometer (Agilent Technologies, Inc., Santa Clara, CA) and identified using the NIST 98 mass spectral library, version 1.7 (National Institute of Standards and Technology, Gaithersburg, MD). Operating conditions for MS were as follows: ion source temperature of 230 °C, electric multiplier tube (EMT) voltage of 2752.9 V, and mass scan range of m/z 36−250 atomic mass units at the rate of 2.76 scans/min. Samples were analyzed in triplicate, and volatile compounds were quantified from peak area measurements compared against the internal standard (ethyl pentanoate). The rate of formation of hexanal in the sample was used as a measure of the rate of oxidation of lipids in MFGM. The other volatiles were quantified in a similar manner using ethyl pentanoate as internal standard.

RESULTS AND DISCUSSION

MFGM Composition. The compositions of freeze-dried and spray-dried MFGM from cheese whey are given in Table 1. The protein content of the freeze-dried MFGM was slightly higher than that of the spray-dried MFGM, whereas the fat content of the spray-dried sample was higher than that of the freeze-dried sample. The reasons for this are not obvious. It is likely that during sampling of the precipitate (which was an inhomogeneous mixture of solid and liquid phases) from the desludger for freeze-drying, more of the solids and less of the liquid phase might have been sampled, resulting in the observed variation in

Table 1. Compositions of Freeze-Dried and Spray-Dried MFGM Samples on a Dry Weight Basis

	spray-dried MFGM	freeze-dried MFGM
moisture (%)	6.03 ± 0.02[a]	4.55 ± 0.01
ash (%)	2.19 ± 0.04	1.81 ± 0.05
fat (%)	19.27 ± 0.32	17.08 ± 0.18
protein (%)	65.56 ± 0.11	73.08 ± 0.16

[a] ± represents standard deviation (n = 6).

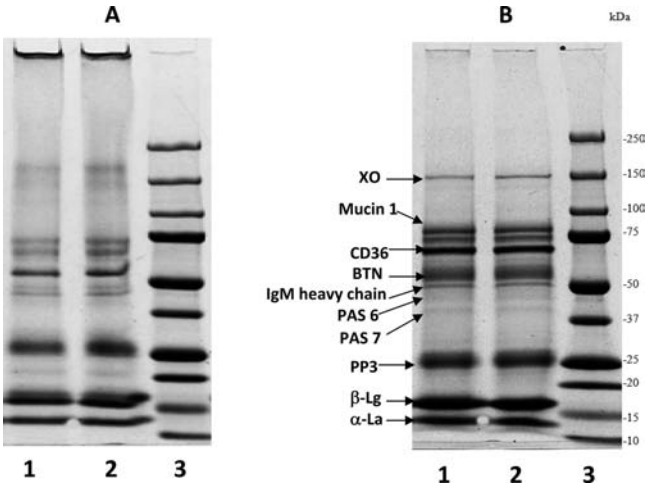

Figure 1. SDS-PAGE of cheese whey-derived MFGM under nonreducing (A) and reducing (B) conditions: sample 1, spray-dried; sample 2, freeze-dried; sample 3, molecular weight markers. Band identification: XO, xanthine oxidase; mucin 1 glycoprotein; CD36, cluster differentiation 36; BTN, butyrophilin; ADPH, adipophilin; PAS6/7, periodic acid Schiff 6/7; PP3, proteose−peptone 3; β-Lg, β-lactoglobulin; α-La, α-lactoalbumin.

composition. Nevertheless, on an average, the MFGM isolated from cheese whey contained 17−19% fat, 66−73% protein, and about 2% ash, the remaining being moisture and lactose. These values are in agreement with those reported in the literature for MFGM.[13,18]

The protein to lipid weight ratio in a typical plasma membrane is about 1:1,[19] and it varies from 1.5 to 4 in some specialized membranes.[20] The protein content of milk fat globule membrane, however, has been reported to be in the range of 25−70% depending on the method of isolation.[13,18,21,22] The MFGM is usually isolated from unpasteurized cream, which involves multiple washings with water or buffer followed by separation of the MFGM from the triglyceride core by churning, and the released MFGM is recovered by centrifugation or salting-out with ammonium sulfate or microfiltration.[18,21] The compositional data presented in Table 1 are the first report on the composition of the MFGM isolated from cheese whey.

Protein Composition of MFGM. Figure 1 shows the SDS-PAGE profile of MFGM proteins under reducing and nonreducing conditions. Under nonreducing conditions, the protein profile showed a high molecular weight polymeric protein that could not migrate into the separating gel (Figure 1A). This polymeric protein species disappeared under reducing conditions with concomitant increase in the intensity of β-lactoglobulin, α-lactalbumin, and other membrane protein bands, notably

Table 2. Fatty Acid Composition of Total Fat in MFGM Isolated from Cheese Whey

	MFGM (%)			MFGM (%)	
fatty acid	freeze-dried	spray-dried	fatty acid	freeze-dried	spray-dried
C4:0	1.95	2.10	C17:1	0.20	0.17
C6:0	1.05	1.13	C18:0	14.65	13.87
C8:0	0.50	0.57	C18:1	25.05	24.60
C10:0	1.30	1.37	C18:1T	4.40	4.40
C12:0	1.80	1.83	C18:2	5.70	7.23
C13:0	0.15	0.17	C18:2T	1.05	0.93
C14:0	7.80	7.73	C18:3	0.50	0.73
C14:1	0.50	0.50	C20:0	0.20	0.20
C15:0	1.00	0.93	C20:3	0.50	0.37
C16:0	27.65	27.43	C20:4	0.60	0.40
C16:1	1.10	1.10	C21:0	0.60	0.50
C16:1T	0.35	0.30	C22:0	0.10	0.10
C17:0	0.65	0.63	C22:5	0.25	0.17

the bands corresponding to 51−81 kDa (Figure 1B). This suggested that a portion of β-lactoglobulin and α-lactalbumin contaminants in MFGM was in the form of disulfide cross-linked polymers. It is also evident that some of the membrane proteins were also in a polymerized state, disulfide cross-linked either with whey proteins or with other membrane proteins. The disulfide-induced polymerization of proteins might have occurred either during pasteurization of milk or during pasteurization of whey.

Apart from β-lactoglobulin and α-lactalbumin, the MFGM contained seven major protein bands corresponding to molecular weights of 150, 81, 75, 70.3, 60−65, 51.6, and 28 kDa. These bands tentatively correspond to xanthine oxidase (150 kDa), cluster of differentiation 36 (CD36) (70.3 kDa), butyrophilin (60−65 kDa), IgM heavy chain (51.6 kDa), and proteose peptone 3 (PP3) (28 kDa).[1,22] The identities of bands corresponding to 75 and 81 kDa are not known. However, the 81 kDa band might belong to either glycoprotein 2 (mucin 1), polymeric Ig receptor, or lactoperoxidase.[22] The bands corresponding to PAS 6 and 7 are very faint. It is likely that these proteins, which are loosely attached to the outside of the MFGM,[1] might have been partially detached during milk homogenization prior to pasteurization and cheesemaking. It is noteworthy that the SDS-PAGE profile did not contain bands corresponding to α-, β-, and κ-caseins, indicating that the MFGM fraction isolated from cheese whey did not contain casein fines as contaminants. Qualitatively, the relative intensities of various MFGM proteins identified in Figure 1B are significantly different from those observed in the SDS-PAGE of MFGM isolated from unpasteurized cream.[1,23] For instance, the CD36 (70.3 kDa) and PP3 (28 kDa) bands in Figure 1B are relatively much more intense than those previously reported for MFGM isolates from cream[23] or buttermilk.[24,25] This might be due to alterations in the MFGM caused by various pretreatments and differences in isolation methods. Nevertheless, the SDS-PAGE profile shows that the MFGM isolated from cheese whey contains proteins that are regarded are bioactive.

Fatty Acid Composition of MFGM. Table 2 shows the fatty acid composition of the total fat in freeze-dried and spray-dried MFGM isolate from cheese whey. The fatty acid compositions of both freeze-dried and spray-dried samples were very similar,

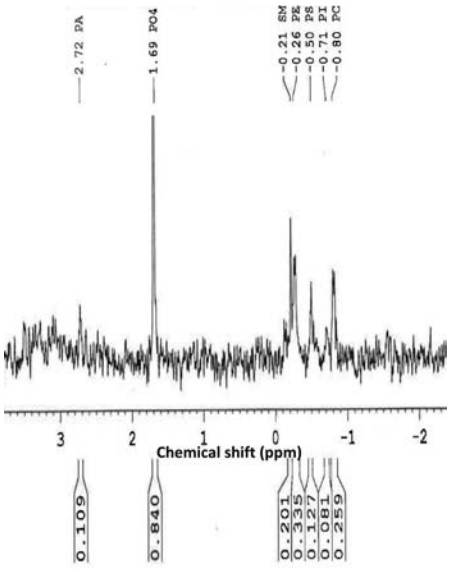

Figure 2. ^{31}P NMR spectrum of phospholipids in freeze-dried MFGM. Peak identification: PA, phosphatidic acid; PO_4, inorganic phosphate; SM, sphingomyelin; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine.

Table 3. Phospholipid Composition of Freeze-Dried MFGM Isolated from Cheese Whey

phospholipid	amount (g/100 g sample)	% of total phopholipids
phosphatidic acid (PA)	0.73	8.15
sphingomyelin (SM)	1.52	17.37
phosphatidylethanolamine (PE)	2.53	28.27
phosphatidylserine (PS)	1.39	15.53
phosphatidylinositol (PI)	0.72	8.04
phosphatidylcholine (PC)	2.06	23.00
total	8.95	

indicating that the drying method did not cause destruction of any of the fatty acids in the samples. The major fatty acids of MFGM lipids were palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids. The content of other unsaturated fatty acids, such as C14:1, C16:1, and C18:2, were very low. These values are comparable to those reported in the literature.[22] Of the total fatty acids, about 26.8% were cis-monounsaturated, 7.5% cis-polyunsaturated, 5.8% trans-fatty acids, and 56% saturated.

Phospholipid Composition. Figure 2 shows the ^{31}P NMR spectrum of the freeze-dried MFGM sample that was used to determine the composition of MFGM phospholipids given in Table 3. Because the exact molecular weights of MFGM phospholipids species are not known, the relative percent distribution of each phospholipid species was calculated on the basis of the molecular weights of soy phospholipids. The phospholipid composition of MFGM reported in Table 3 is different from that reported by Fong et al.;[22] however, it is similar to the values reported by Burling et al.,[26] especially the phosphatidylserine content. In the former case, the phosphatidylserine content was found to be only about 5% as compared to about 15% found in this study and 12% reported by Burling et al.[26] This discrepancy, as the authors[22] had acknowledged, might be related to the nonlinear response of the evaporative light scattering detector (ELSD) used for quantification of the elution peaks of phospholipid species. Fong et al.[22] reported 31% phosphatidylcholine content in MFGM derived from buttermilk, which is significantly higher than the 21% found in this study. In addition, the MFGM from cheese whey contained about 8% phosphatidic acid, whereas it was not found in MFGM isolated from cream.[22,26] Taken together, it appears that during various treatments leading to cheese manufacture, about 8% of phosphatidylcholine is converted to phosphatidic acid by the action of either endogenous or starter culture-derived phospholipase-D.

It should be noted that the total phospholipid content of the freeze-dried MFGM isolate was about 9% (Table 3), whereas the total lipid content was about 17% (Table 1). This suggests that the neutral lipid content was about 8% or about 47% of the total lipids. The triglyceride content of MFGM has been reported to be in the range of 20−80% of the total depending on the method of isolation,[27] and recently Fong et al.[22] reported a value of 53%. Thus, the neutral lipid to phospholipid ratio of the MFGM isolated from cheese whey appears to be similar to the MFGM isolated from cream.

Phase Transitions of MFGM Lipids. Phase transition of membrane lipids from a crystalline state to liquid state generally depends on the microstructure of the membrane. Different processing methods employed might alter this microstructure and thereby the thermal properties and presumably the oxidative stability of MFGM. To understand this phenomenon, the morphological properties and thermal properties of freeze-dried and spray-dried MFGM samples were studied.

Figure 3 shows the microscopic images of spray-dried and freeze-dried MFGM samples. Morphologically, these two preparations were very different: The freeze-dried MFGMs were irregular flaky translucent sheets with sharp edges (Figure 3A), whereas the spray-dried MFGMs were spherical particles of about 100 μm in diameter (Figure 3B). When the spray-dried MFGM was dissolved in water and then freeze-dried, the MFGM particles appeared as fragmented rods and sheets (Figure 3C) much smaller in size vis-a-viz the freeze-dried only MFGM sample (Figure 3A). It appeared that the high temperature and high shear conditions used in spray-drying caused melting and tranformation of large lamellar membrane sheets into smaller fragments and rod-shaped cylindrical micelles.

The DSC thermograms of freeze-dried and spray-dried MFGM, shown in Figure 4, reveal marked differences in the melting temperature profile. The freeze-dried MFGM exhibited major endothermic peaks at 37.8 and 75.6 °C and minor shoulders at 45.1 and 66.4 °C (Figure 4A). During subsequent rescanning of the sample after cooling to 5 °C (curves 2−5), an endothermic peak at 35.6 °C reappeared, but the shoulder at 66.4 °C and the peak at 75.6 °C have disappeared, suggesting that the latter two transitions were irreversible and might represent denaturation temperatures of proteins in the MFGM. It is known that α-lactalbumin and β-lactoglobulin exhibit thermal transition temperatures at 64−66 and 72−76 °C, respectively.[28] It is likely that the transition at 66.4 °C and the peak at 75.6 °C might belong to these two proteins in the MFGM samples. The reappearance of an endothermic peak at 35.6 °C during multiple scans indicates that this transition belongs to reversible phase transition of MFGM lipids.

The DSC thermogram of spray-dried MFGM contained three endothermic transitions at 48, 66.1, and 75.5 °C (Figure 4B,

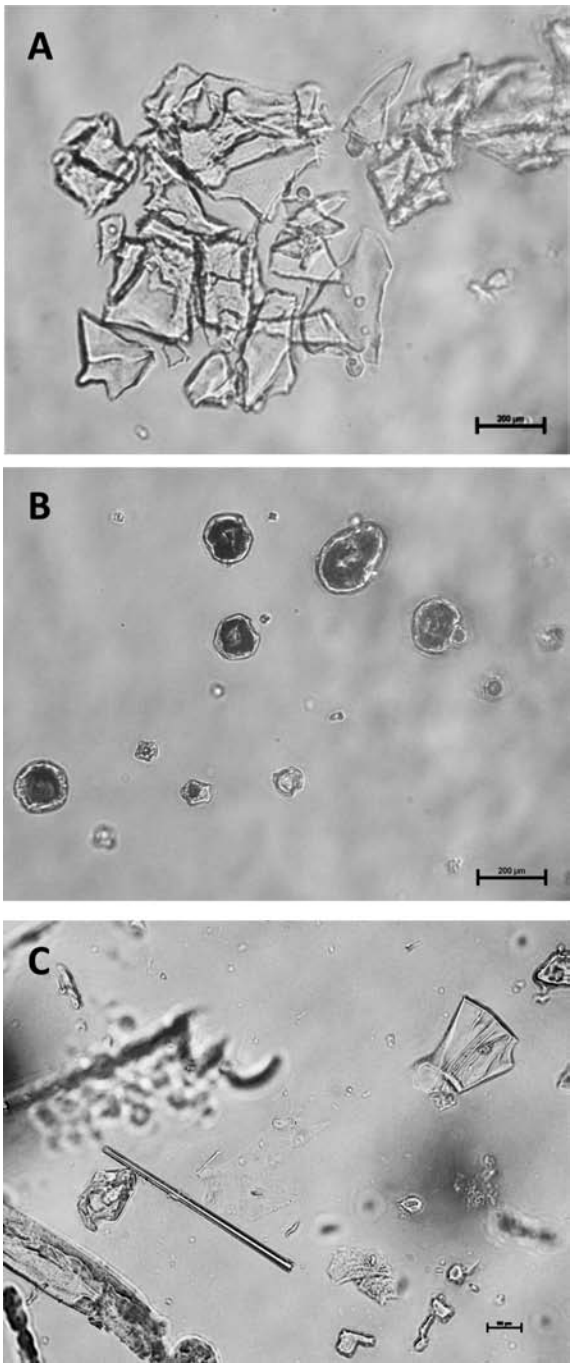

Figure 3. Light microscopic images of freeze-dried (A), spray-dried (B), and spray-dried sample dissolved in water and then freeze-dried (C) MFGM particles. The bar represents 200 μm for panels A and B and 100 μm for panel C.

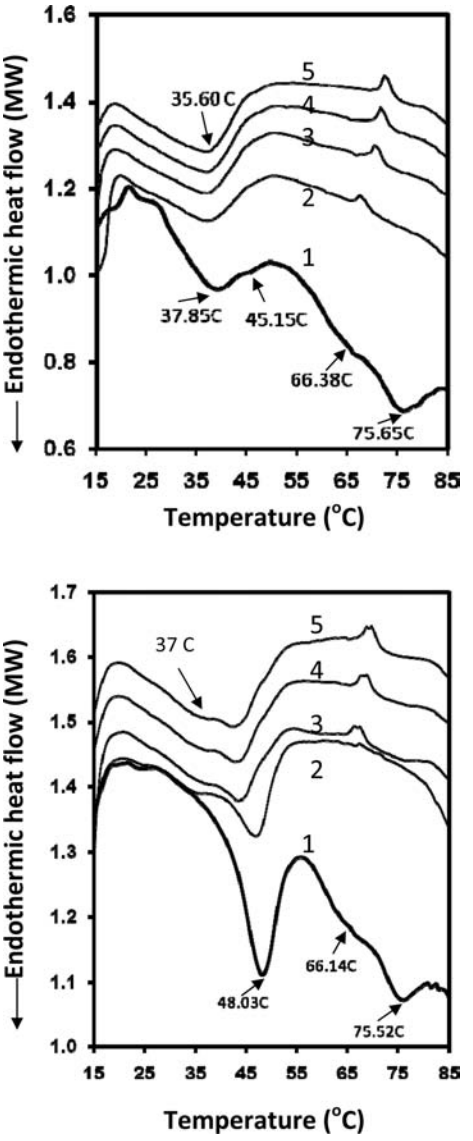

Figure 4. Differential scanning calorimeter thermogram of freeze-dried (A) and spray-dried (B) MFGM. The numbers on the curves represent the first, second, third, fourth, and fifth rescans of the same sample. The melting termperature (T_m) of various species are indicated.

curve 1). During rescanning of the sample, the transitions corresponding to 66.1 and 75.5 °C have disappeared and a shoulder at about 35−37 °C and a peak at 47 °C reappeared (Figure 4B, curve 2). This again confirms that the transitions at about 66.4 and 75.6 °C might belong to α-lactalbumin and β-lactoglobulin, respectively, which were irreversibly denatured during the first scan. During repeated scanning of the sample, the transition at 47 °C progressively moved toward lower temperature and the relative magnitude of the transition at 35−37 °C increased. The reappearance of peaks at 35−37 and 45−47 °C indicates that these were reversible transitions of MFGM lipids.

In addition to the endothermic transitions in the 35−37 °C region, an exothermic transition appeared in both freeze-dried and spray-dried samples at 65−70 °C during multiple rescanning of the samples, and the intensity of this peak increased with the number of rescans (Figure 4). This exothermic peak might be related to protein aggregation.

It should be noted that the phase transition temperature of lipids in the freeze-dried and spray-dried MFGM samples were quite different: Whereas the major lipid phase transition in the freeze-dried sample occurred at about 37−38 °C, it was at 48 °C in the spray-dried sample. Several reports in the literature indicate that the typical phase transition temperature of lipids in biological cell membranes is about 37−38 °C,[29,30] which agrees well with the thermotropic phase behavior of freeze-dried MFGMs, which are morphologically in the form of intact lamellar

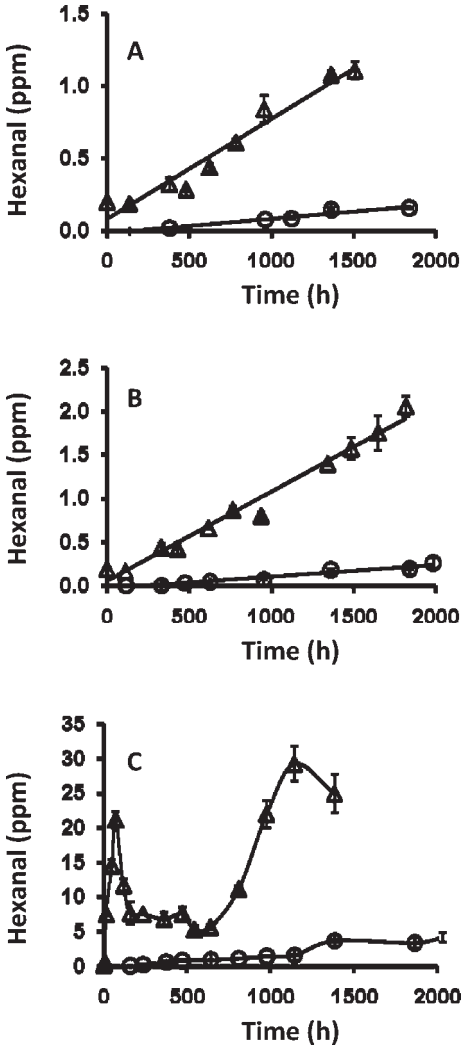

Figure 5. Time evolution of hexanal production in freeze-dried (○) and spray-dried (△) MFGM stored at 25 °C (A), 35 °C (B), and 45 °C (C). (Qunatification of hexanal was based on the amount of ethyl pentanoate as internal standard.)

sheets (Figure 3). The 10 °C shift in the phase transition temperature of lipids in the spray-dried MFGM compared to freeze-dried MFGM suggests that spray-drying had caused major changes in the microstructure of membrane lipids. Although the nature of this microstructural change is unclear, it might be related to altered protein−lipid interactions in the membrane environment, changes in the packing order of lipids, and/or changes in the lateral organization/distribution of the lipids. The fact that a shoulder at 35−37 °C appeared and grew in intensity at the expense of a decrease in the intensity of the transition at 48 °C during repeated scanning of the spray-dried MFGM (Figure 4B) tentatively implicates progressive restoration of the lateral distribution pattern of lipids to a state more comparable to that of the freeze-dried MFGM.

Oxidative Stability of MFGM Lipids. To elucidate if alternations in the morphophological properties and thermotropic phase behavior of MFGM lipids affect their susceptibility to oxidation, we studied the kinetics of formation of hexanal during storage of freeze-dried and spray-dried MFGM samples. The time evolution of hexanal production in freeze-dried and spray-dried MFGM during storage at 25, 35, and 45 °C is shown in Figure 5. Although lipid oxidation generates several volatile compounds, hexanal production is used here as a measure of the rate of oxidation of MFGM lipids. The zero-order rate constant at various temperatures is given in Table 4. At 25 and 35 °C the formation of hexanal increased linearly with time in both freeze-dried and spray-dried MFGM. However, at 45 °C, whereas the formation of hexanal was linear with time in the freeze-dried MFGM, there was a spike in hexanal formation in the spray-dried MFGM at 0−72 h, followed by a lag time and a second phase of hexanal production (Figure 5C). This bimodel formation of hexanal might suggest that the spray-dried sample might contain particles with two different size distributions. The smaller particles with high surface area-to-volume ratio might be more susceptible to oxidation than the larger particles with low surface area-to-volume ratio.

Table 4. Zero-Order Rate Constant of Lipid Oxidation As Determined by Hexanal Production in Freeze-Dried and Spray-Dried MFGM Samples

	zero-order rate constant (ppm/h)	
temperature (°C)	freeze-dried MFGM	spray-dried MFGM
25	9.9×10^{-5}	6.92×10^{-4}
35	1.34×10^{-4}	1.02×10^{-3}
45	2.10×10^{-3}	0.2992 (initial rate, 0−72 h)
		0.0425 (final rate, 544−1144 h)

The relative concentrations of various volatiles formed in the spray-dried MFGM sample during storage at 45 °C are shown in Table 5. Hexanal was the major product of oxidative degradation of MFGM lipids. Alkanes, such as undecane, dodecane, and tridecane, were also found to be present in significant amounts in the headspace volatiles. At all temperatures studied, the rate of generation of hexanal was about 1−2 orders of magnitude greater in the spray-dried MFGM than in the freeze-dried MFGM. This difference in the oxidation rate might arise from an increase in surface area of membrane particles resulting from breakdown of large lamellar sheets into smaller fragments and rod-shaped micelles during spray-drying. Even though the spray-dried MFGM appeared as spherical particles (Figure 3B), these particles seemed to be aggregates of smaller sheets and cylindrical particles with large exposed surface area (Figure 3C). It is unclear whether or not the greater susceptibility of spray-dried MFGM than of the freeze-dried MFGM to oxidation is also in some way linked to differences in thermotropic phase behavior of these samples.

The results presented here clearly show that the MFGM isolated from cheese whey is a rich source of bioactive lipids (i.e., phosphatidylserine and sphingomyelin) and proteins (i.e., CD36, butyrophilin, xanthine oxidase, and mucin 1). It can be used as such as a concentrated source of these milk-derived bioactive components. The cheese-derived MFGM contained about 17−19% lipids on a dry weight basis, of which about ∼50% were phospholipids. Because the MFGM from cheese whey is a byproduct of a process designed to produce fat-free whey protein isolate and concentrate,[14] its potential availability in large volume might make it possible to use it as a source of dairy lecithin. The results presented here also clearly demonstrate that spray-drying alters the morphological state and thermotropic phase behavior of lipids in MFGM and renders them more susceptible to

Table 5. Relative Concentrations of Various Volatile Compounds Formed in Spray-Dried MFGM at Various Incubation Times at 45 °C[a]

compound	% area at 0 day	% area at 3 days	% area at 23 days	% area at 48 days
2-butanone	0.1051	0.3449	1.0529	0.7108
pentanal	0.9193	1.5205	1.3917	1.3262
1-pentene	0.2115	0.4569	0.572	0.7034
hexanal	13.3346	21.4911	13.6617	12.4455
1-butanol	0	0	0.37	0
1-hexanol	0.1787	0.2573	ND	0.3386
2-heptanone	0.3068	0.5835	2.0501	2.0924
pentanoic acid, ethyl ester	39.8538	15.2265	8.5543	15.8802
benzaldehyde	0.9444	1.698	4.1682	4.1984
1-octen-3-ol	0.6308	0.8843	1.6361	1.4362
3-octen-2-one	0.7024	0.8365	0.7242	0.5613
undecane	3.4553	1.6426	0.8961	1.0858
dodecane	5.1824	2.0583	0.7128	1.0231
butanal	0.0981	0.1883	ND	0.0661
2-octanone	0	ND	1.3782	ND
furan, 2-pentyl-	0.3932	0.4858	ND	1.5433
1-heptanol, 4-methyl-	0	ND	0.8217	ND
octanal	0.5371	0.645	ND	0.7102
tridecane	4.3719	1.3983	ND	0.715
cyclopentane, 1,3-dimethyl-, *trans*-	0	0.2078	0	0
2,3-dimethylaziridine	0.2438	ND	0.357	0.2419
benzaldehyde, 4-methyl-	0.2224	ND	0.512	0.4058
3,5-octadien-2-one	0.7029	ND	1.1138	0.8609
acetophenone	ND	ND	0.2025	0.1547
pentane, 1-nitro-	ND	0.3232	0.4404	0.5133
3,5-octadien-2-one, (*E*,*E*)-	ND	0.5944	0.4711	0.2681
2-nonanone	ND	ND	0.3416	0.5228
trichloromethane	ND	ND	0.9375	0.9284
bicyclo[4.2.0] octa-1,3,5-triene	0.5065	0.5299	ND	ND
1-hexanol, 2-ethyl-	0.3207	0.3513	ND	ND
3,5-octadien-2-one, (*E*,*E*)-	0.434	0.7382	ND	ND
cycloheptane	0	0	0.8758	0
nonanal	1.2759	1.6046	ND	ND
propanal, 2-methyl-	0	0.1069	ND	0.1047
butanal, 3-methyl-	ND	ND	ND	0.23
1-tridecene	ND	ND	0.5332	0.5846
2-octenal, (*E*)-	ND	ND	0.2484	ND
2-decenal, (*E*)-	ND	ND	ND	0.2453
2-undecenal	0	0.3069	ND	ND
2-octenal, (*E*)-	0.2903	ND	ND	ND
undecane, 4,6-dimethyl-		ND	0.2993	ND
nonadecane	0.1413	ND	ND	ND

[a] ND, not detected.

oxidation during storage compared to freeze-drying. It is recommended that to preserve the bioactive components from oxidative degradation and to retain the functional food value of MFGM, freeze-drying might be better than spray-drying.

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