Partition of milk phospholipids during ice cream manufacturing

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ABSTRACT

The distribution of phospholipids (PL) within the fat and serum phase of ice cream manufacturing was evaluated through partition coefficients ($K_{PL}$) after mixing, pasteurization, freezing, and hardening. Ice creams containing about 40.41 ± 3.45 (± standard deviation; control formulation) and 112.29 ± 9.06 (enriched PL formulation) mg of PL per g of fat were formulated with nonfat dry milk and β-serum, respectively. Overall, the $K_{PL}$ were lower than 1, indicating that the PL were predominantly found in the fat phase, and only a small amount was left in the serum and sediment. Confocal micrographs visually confirmed this generalization. The addition of PL significantly increased the viscosity of the mixes between 4- and 9-fold, depending on the shear rate. Additionally, mixes containing high PL exhibited higher yield stress than those formulated with low PL (0.15 ± 0.09 and 0.016 ± 0.08 Pa, respectively). Ice creams with high PL delayed the onset of meltdown and exhibited a slower rate of a meltdown than low-PL ice creams (18.53 ± 0.57 and 14.83 ± 0.85 min, and 1.01 ± 0.05 and 0.71 ± 0.04% min⁻¹, respectively). This study provides useful guidelines for manufacturing ice cream enriched in milk PL. Additionally, the use of β-serum, a byproduct stream, as a source of PL is illustrated. The development will require studying the sensorial description of the product as well as consumer acceptance.

Key words: beta-serum, byproduct utilization, ice cream, meltdown

INTRODUCTION

The unique composition and structure of the milk fat globule membrane (MFGM) provide biological functions, bioactive properties, and technological opportunities (Jiménez-Flores and Brisson, 2008; Jukkola et al., 2019). The globule membrane is made of a mixture of glycosylated proteins, phospholipids (PL), choline, and cholesterol arranged in a complex structure of an inner monolayer of phospholipids and proteins, an electron-dense proteinaceous coat, and bilayer membrane of phospholipids and proteins (Dewettinck et al., 2008; Brink and Lönnerdal, 2020). An in-depth discussion of the biological functions of MFGM can be found elsewhere (Lopez, 2011; Raza et al., 2021).

Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM) are the predominant phospholipids found in MFGM (Ortega-Anaya and Jiménez-Flores, 2019). These PL are relevant to human nutrition because they provide several bioactivities, including beneficial effects on cognitive performance (Hellhammer et al., 2010), therapeutic ability in experimental arthritis in rats (Hartmann et al., 2010), anticancer activity of colon cancer cells (Kuchta-Noctor et al., 2016), antioxidant activity in vitro assays (Huang et al., 2020a), protection against gastrointestinal infections (Küllenberg et al., 2012), and enhanced digestive properties of infant formula (Lopez et al., 2015). The term bioactive broadly refers to molecules that can provide health benefits beyond basic nutrition (Biesalski et al., 2009). The bioactivity of PL derived from MFGM has been recently reviewed elsewhere (Ortega-Anaya and Jiménez-Flores, 2019).

In addition to the health benefits, PL derived from MFGM provide technological opportunities, such as improvements in the texture of bakery goods (Huang et al., 2019), stability of liposomes (Thompson et al., 2006), and oil-in-water emulsions (Phan et al., 2016). Milk fat globule membrane is concentrated to produce enriched PL ingredients (Rathnakumar et al., 2021a). These concentrates of PL (20 to 70%) are derived from byproduct streams, including buttermilk (BM), whey protein phospholipid concentrate (WPPC), whey but-
termilk (WBM), and β-serum (βS) (Raval and Mistry, 1999; Sodini et al., 2005; Camacho Flinois et al., 2019). Huang et al. (2020b) reviewed the current industrial practices for concentrating PL from dairy streams. Research in the utilization of βS is not as advanced as BM and WPPC, and there are opportunities for commercial applications for βS as enriched PL ingredients. Industrially, βS is the byproduct derived from the manufacture of anhydrous milk fat after the inversion of concentrated cream, and it is characterized by higher protein content than buttermilk and WPPC (Rathnakumar et al., 2021b). Overall, the gross composition of βS resembles that of nonfat dry milk (NFDM) except for the high content of PL (8%–10% on a dry basis; Rathnakumar et al., 2021a,b). Such concentration of PL may improve the emulsification during ice cream. Proteins and emulsifiers create an interfacial space that is affected during the manufacture of ice cream. Moreover, stabilizers serve multiple purposes, such as improving the emulsion stability, stabilizing air bubbles, reducing the growth of lactose crystals, and preventing the migration of free water. In this investigation, we evaluated the feasibility of replacing NFDM with βS during the manufacture of ice cream. The objectives of this work are to evaluate the partition of PL within the manufacture of ice cream (mixing, pasteurization, freezing, hardening, and melted ice cream) and to evaluate the impact of PL content on selected properties of ice cream mix (e.g., viscosity, particle size, gel electrophoresis) and end product (e.g., hardness, overrun, and melting behavior).

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Experimental Plan

The experiments were designed to evaluate 3 major areas. Study 1 consisted of monitoring the partition of PL at different locations throughout the manufacturing of ice cream, including mixing, pasteurization, freezing, and hardening (Figure 1). Study 2 evaluated the effect of PL content on selected quality parameters of ice cream mix (ICM; flow curve, particle size, zeta potential, and gel electrophoresis). Study 3 assessed the effect of PL on the hardness and melting characteristics of the resulting ice cream.

Formulation and Composition

A standard ice cream formulation was used to evaluate the partition of PL through the manufacture of ice cream as well as the effect of PL content on selected properties of ice cream mix and end product. Ice cream mixes were formulated to contain 40.0% total solids, 11.0% fat, 4.5% protein, and 40.41 ± 3.45 or 112.29 ± 9.06 mg of PL·g⁻¹ of total fat (Table 1). Ice cream ingredients, such as heavy cream, skim milk, NFDM, granulated sugar, and dry corn syrup, were purchased.

Figure 1. Schematic diagram of the manufacturing step of ice cream. Samples were obtained after (1) mixing, (2) pasteurization, (3) batch freezing, (4) hardening, and (5) ice cream.
from a local supermarket (Albertsons, Las Cruces, NM), the blend of stabilizers was obtained from a commercial vendor (Continental Colloids Inc., West Chicago, IL), and βS was obtained from a regional cheese factory (Valley Queen, Milkbank, SD). Upon reception, the βS was freeze-dried with a batch drier (HarvestRight, Salt Lake City, UT). Mixes containing 40.41 ± 3.45 mg of PL·g⁻¹ of total fat were formulated with NFDM (no βS), whereas βS (no NFDM) was used to formulate mixes containing about 112.29 ± 9.06 mg of PL·g⁻¹ of total fat. The mixes were denominated as low-PL ice cream mixes and high-PL ice cream mixes (LPL-ICM and HPL-ICM, respectively).

All ICM were analyzed for total solids, protein, fat, and pH. The guidelines reported by AOAC International were followed during the analysis of total solids (method 941.08), total protein (method 990.20), and total fat (method 952.06; AOAC International, 2000). The pH of the mixes was measured in 75 mL using an Orion pH meter (Versa Star Pro, Thermo Fisher Scientific, Waltham, MA).

## Ice Cream Manufacture

The formulation and mixing of the ice cream ingredients were carried out according to the methodology reported by Sim et al. (2021). Briefly, dry ingredients were dissolved in skim milk and heavy cream at room temperature for 15 min with a laboratory blender (Polytron, CH-6010, 60 Hz, Switzerland). The formulated mixes were then pasteurized at 85°C for 15 s in a continuous Armfield unit (FT74 UHT/HTST, Ringwood, Hampshire, UK) with a tubular heat exchanger. Then, the ICM was aged at 6°C for 12 h. Afterward, batches of pasteurized mixes (600 mL) were frozen in a batch freezer (Smart Scoop BC1600XL, Breville, Australia) until the temperature in the mix was about −7°C (approximately 15 min). After freezing, the frozen samples were packaged in 150-mL plastic cups and subsequently hardened at −40°C for 8 h. Then, the samples were transferred to a conventional freezer (−18°C) and stored for a week before analysis.

## Partition of Phospholipids

The partition of PL was evaluated through the partition coefficient ($K_{PL}$) that is defined as the ratio of PL in the serum and sediment to the PL in the fat phase, according to Equation [1]:

$$K_{PL} = \frac{[PL]_{serum+sediment}}{[PL]_{fat}}. \quad [1]$$

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LPL-ICM</th>
<th>HPL-ICM</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>40.95 ± 0.17a</td>
<td>40.54 ± 0.51a</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>11.33 ± 1.04a</td>
<td>10.55 ± 1.42a</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>4.55 ± 0.57a</td>
<td>4.75 ± 0.62a</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>0.79 ± 0.04a</td>
<td>0.83 ± 0.03a</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>23.44</td>
<td>24.41</td>
</tr>
<tr>
<td>pH</td>
<td>6.58 ± 0.02a</td>
<td>6.59 ± 0.03a</td>
</tr>
<tr>
<td>Total phospholipids (mg/fat)</td>
<td>40.41 ± 3.45a</td>
<td>112.29 ± 9.06b</td>
</tr>
</tbody>
</table>

*Means ± SD (n = 3) within columns with different superscripts indicate significant differences (P < 0.05) according to the Tukey test.

The total PL from the ICM were quantified after mixing, pasteurization, freezing, and hardening (Figure 1). Samples (~10 mL) collected at different stages were centrifuged (Jouan CR412, Jouan Inc.) at 4,000 × g for 30 min at −9°C to separate the fat phase from the serum and sediment phase. The serum and sediment were considered one phase, namely the serum phase. The PL in each phase were extracted following the methodology reported by Cheng et al. (2019). Samples of 2 g were mixed with 20 mL of chloroform:methanol solution (2:1, vol/vol). The mixture was vortexed for 3 min, followed by centrifugation at room temperature (4,200 × g) for 20 min. The methanol phase was discarded, and the chloroform phase was transferred to a test tube, where the chloroform was removed at 45°C using a vacuum oven. The extracted lipids were fractionated to recover the PL through solid-phase microextraction using an activated silica gel column (1 cm × 10 cm). Samples of dried lipids (0.1 g) were dissolved in 1 mL of chloroform: methanol solution (95:5, vol/vol) and run through the previously conditioned column with 10 mL of the chloroform:methanol solution. The PL were recovered with 10 mL of methanol and 10 mL of chloroform: methanol: water (5:3:2 vol/vol). Finally, solvents were evaporated at 40°C under vacuum, and the total phospholipids were calculated using Equation [2]. The extracted PL were stored at −20°C until further analysis.

$$\text{Total phospholipids(%) = } \frac{\text{weight of dried fraction}}{\text{weight of lipids}} \times 100. \quad [2]$$

In addition to the quantification of total PL, microstructure images of the serum and solid phase were determined by confocal laser scanning microscopy (CLSM) using a Leica microscope (TCS SP5 II, Leica, Wetzlar, Germany), according to the methodology reported by Sim et al. (2021). Briefly, dry ingredients were carried out according to the methodology reported by Cheng et al. (2019). Samples (~10 mL) collected at different stages were centrifuged (Jouan CR412, Jouan Inc.) at 4,000 × g for 30 min at −9°C to separate the fat phase from the serum and sediment phase. The serum and sediment were considered one phase, namely the serum phase. The PL in each phase were extracted following the methodology reported by Cheng et al. (2019). Samples of 2 g were mixed with 20 mL of chloroform:methanol solution (2:1, vol/vol). The mixture was vortexed for 3 min, followed by centrifugation at room temperature (4,200 × g) for 20 min. The methanol phase was discarded, and the chloroform phase was transferred to a test tube, where the chloroform was removed at 45°C using a vacuum oven. The extracted lipids were fractionated to recover the PL through solid-phase microextraction using an activated silica gel column (1 cm × 10 cm). Samples of dried lipids (0.1 g) were dissolved in 1 mL of chloroform: methanol solution (95:5, vol/vol) and run through the previously conditioned column with 10 mL of the chloroform:methanol solution. The PL were recovered with 10 mL of methanol and 10 mL of chloroform: methanol: water (5:3:2 vol/vol). Finally, solvents were evaporated at 40°C under vacuum, and the total phospholipids were calculated using Equation [2]. The extracted PL were stored at −20°C until further analysis.

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reported by Rathnakumar et al. (2021b). Briefly, the phospholipids were stained with a chloroform solution containing 0.01% Rd-dope (Avanti Polar Lipids Inc., Alabaster, AL). Then, 20 µL of the dissolved PL was transferred to a test tube and mixed with the fluorescent dyes to a ratio of 1:100 (vol/vol) for 20 min in the dark. Then, a droplet of the stained sample was placed in a microscope slide and dried at atmospheric conditions. The excitation of the Rd-dope was achieved at 559 nm, using emission from a diode laser. All images were acquired at room temperature.

**Ice Cream Mix Analysis**

**Flow Curve.** The flow curve of the ICM was determined in a rheometer (Discovery Hybrid rheometer, HR 30, TA instruments, New Castle, DE) equipped with a 20-mm parallel plate and a Peltier steel plate (108783). Flow measurements were carried out within a shear rate range of 1 to 100 s⁻¹, recording 10 data points per test. The measurements were determined at 4°C. The viscosity (η) of the ICM as a function of shear rate (γ) was represented with the Herschel-Buckley model (Equation [3]) (Ranaweera et al., 2022).

\[
η = η_o + K \cdot γ^{(n-1)},
\]

where \( η_o \) is the yield stress (Pa), \( K \) is the consistency index (Pa∙sⁿ), and \( n \) is the flow behavior index.

**Particle Size Distribution and Zeta Potential.** A ZetaSizer Nano ZS (Malvern Instruments Ltd., Cambridge, UK) was used to determine the particle size distribution and zeta potential of ICM, following the methodology described elsewhere (Rathnakumar et al., 2021a). Mixes were brought to room temperature and equilibrated for 10 min before the measurements. Aliquots of 10 µL were transferred to disposable cuvettes (DTS 0012, Sigma-Aldrich, St. Louis, MO) and diluted 1,000× with deionized water. Afterward, the cuvettes were transferred into the measuring chamber, where a scattering angle of 173° and a refractive index of 1.46 was used to measure the distribution of particles. Average particle size and their relative distribution were obtained from the percentage of volume versus droplet diameter graph. The zeta potential analysis was carried out in disposable polycarbonate cuvettes (ATA Scientific, DTS1061), and the measurements were repeated at least 10 times per run.

**Gel Electrophoresis.** The protein profile of the ice cream mixes, ice cream, and dripped portion of melted ice cream was determined using SDS-PAGE under reducing conditions as reported by Rathnakumar et al. (2021b). Five milliliters of sample was mixed with 20 mL of cold acetone (−20°C) to precipitate the proteins. After 60 min, the solution was centrifuged (Jouan CR412, Jouan Inc., Winchester, VA) at 3,600 × g for 20 min at 0°C. The precipitated proteins were recovered and dissolved in 2 mL of PBS. Aliquots of 5 µL of the dissolved proteins were transferred into a vial containing 4.75 µL of 2× Laemmli sample buffer (Bio-Rad, Hercules, CA) and 0.25 µL of 4% 2-mercaptoethanol (Fisher Scientific, Hampton, NH). Dissolved proteins were heated at 90°C for 5 min and cooled down to room temperature. Afterward, 10 µL of the preparation was loaded into Tris-acrylamide gels (4–15% Mini-Protean TGX precast gels with 10 wells, Bio-Rad). Gels were run for 1 h at 200 V using Tris/Glycine/SDS buffer (Bio-Rad). Then, the gels were removed and stained using Bio-safe Coomassie G-250 stain (Bio-Rad) to yield the protein pattern. The individual proteins were estimated based on the molecular weight using a standard from Bio-Rad (precision plus protein standards, 161–0375). The obtained gels were analyzed qualitatively by visual observation of the protein bands.

**Ice Cream Analysis**

**Fat Destabilization.** Measurements of fat destabilization (\( F_d \)) were determined as the percentage of turbidity in ice cream with respect to the turbidity in ICM (Adapa et al., 2000). Turbidity measurements were carried out in a UV-spectrophotometer (Genesys 10 UV-VIS mode #840–208200, Thermo Scientific) set at 540 nm. Fat destabilization was calculated according to Equation [4]:

\[
F_d = \frac{\text{turbidity of ice cream}}{\text{turbidity of ice cream mix}} \times 100.
\]

**Overrun.** The amount of air incorporated within the ice cream was expressed as the percentage of overrun, according to the methodology reported by Muse and Hartel (2004). A fixed volume of 100 mL was used to compare the weight of the ICM and ice cream using Equation [5]:

\[
\text{Overrun} = \frac{\text{weight of ice cream mix} - \text{weight of ice cream}}{\text{weight of ice cream}} \times 100.
\]

**Hardness.** The hardness of ice cream stored at −20°C for a week was determined with a TA.XT Plus Texture Analyzer (Texture Technologies Corp. and Stable Micro Systems Ltd., Hamilton, MA). Individual cups of ice cream (150 g) were placed on the platform...
of the texturometer and immediately assessed for hardness. The test was conducted at room temperature (25°C), and samples were vertically penetrated with a cylindrical probe TA-55 20 mm diameter) to a depth of 20 mm at a speed of 2 mm∙s⁻¹.

**Melting Behavior.** Ice cream samples were evaluated for melting behavior using oscillatory analysis with an MCR92 rotational rheometer (Anton Paar USA Inc., Vernon Hills, IL) coupled with a Peltier chamber with a plate-plate geometry (25 mm diameter). Details on the methodology can be found elsewhere (Sim et al., 2021). The oscillatory analysis was conducted within a temperature range of −20°C to 10°C, at a heating rate of 0.5°C min⁻¹, a deformation amplitude of 0.5%, a frequency of 10 Hz, and a gap width between plates of 3 mm. The storage module (G') and the loss module (G'″) were recorded continuously during the analysis. Moreover, the melting curve was also evaluated using the loss tangent \( \tan(\delta) = \frac{G'″}{G'} \).

**Meltdown.** The meltdown characteristics of ice cream were determined following the methodology reported by Muse and Hartel (2004). Samples of ice cream (about 70 g) were removed from the plastic cups and placed in a wire mesh (0.833 mm) mounted on a beaker at room temperature. The weight of the dripped ice cream was recorded every 5 min to obtain the onset, rate, and maximum melt. The dripped portion was analyzed to determine the total phospholipids, microstructure, and PL profile.

The quantification of the PL profile was determined using a UHPLC system (Dionex Ultimate 3000, Thermo Scientific) coupled to a charged aerosol detector (CAD, DionexCorona Veo RS, Thermo Scientific), according to the methodology reported by Rathnakumar et al. (2021a). The 5 major phospholipids were identified by their retention time and were quantified by comparing their peak area with a standard curve. The relative distribution of PL was calculated using Equation [6]:

\[
\text{relative distribution} = \frac{\text{concentration of individual PL}}{\text{concentration of total PL}}.
\]

**Statistical Analysis**

The manufacture of ice cream and its analysis was performed in triplicate, and the results were expressed as the mean ± standard deviation. A pairwise comparison was used to analyze the \( K_{PL} \) between treatments and levels via 2-way ANOVA using the Holm-Sidak test. All figures were made using SigmaPlot software V14.5 for Windows (SPSS Inc., Chicago, IL).

**RESULTS AND DISCUSSION**

**Partition Coefficient**

The partition coefficient of PL \( K_{PL} \) at selected locations within the manufacture of ice cream is given in Figure 2. Overall, the \( K_{PL} \) values ranged from 0.13 to 0.31 and 0.07 to 0.49 for LPL-ICM and HPL-ICM, respectively, and gradually increased with the processing steps. Partition coefficients describe the distribution of a solute between 2 immiscible phases (Speight, 2018). Values of \( K_{PL} \) lower than 1 indicate that the PL were predominantly found in the fat phase, and only a small amount was left in the serum and sediment. This generalization was visually exemplified by confocal micrographs (Figure 3), regardless of the sample. Abundant droplets of PL were observed in the fat phase (Figure 3a–d and 3i–l), while few droplets were observed in the serum (Figure 3e–h and 3m–p).

After mixing, the \( K_{PL} \) values for HPL-ICM were significantly lower than the LPL-ICM (0.07 ± 0.02 and 0.13 ± 0.01, respectively, Figure 2). Ice cream mixes are colloidal suspensions containing fat droplets coated with a protein-emulsifier layer (Goff, 1997). Phospholipids are regarded as a natural emulsifier that can be used in several food formulations (Rombaut et al., 2007). The high concentration of PL (~112 mg of PL∙g⁻¹ of fat) added into the HPL-ICM along with the mechanical energy provided during mixing may lower the interfacial tension between the fat and the water phase, creating larger fat droplets surrounded by PL than the mixes with lower PL concentration (~40 mg of PL∙g⁻¹ of fat). Indeed, confocal images showed relatively large droplets of PL in the fat phase of HPL-ICM, spanning from about 20 to 90 µm (Figure 3i). Contrary, relatively small droplets of PL (15–50 µm) were observed in the fat phase of LPL-ICM (Figure 3a). The size range of PL droplets after mixing was much larger than the PL droplets in freeze-dried βS, 2 to 5 µm (Rathnakumar et al., 2021a), and native droplets in MFGM, 5 to 10 µm (Gallier et al., 2010). Such differences in size can be attributed to droplet agglomeration during mixing.

For both samples, a maximum value of \( K_{PL} \) was observed after freezing (0.29 ± 0.01 and 0.46 ± 0.02 for LPL-ICM and HPL-ICM, respectively, Figure 2) without significant changes afterward. The gradual increment of the \( K_{PL} \) values during the processing steps suggests that some migration of PL occurred from the emulsion into the serum, possibly due to the mechanical damage of the emulsion during pasteurization and freezing (Hansen et al., 2020). Corredig and Dalgleish (1998) reported that the solubility and the emulsifying properties of MFGM were negatively affected during pasteurization. The migration of PL droplets from the emulsion to the serum
and sediment was illustrated in the confocal micrograph (Figure 3b, 3f, 3j, and 3n). Emulsions within the fat phase are subjected to extensive whipping and the formation of ice crystals during freezing, forming a network made of partially coalesced and agglomerated fat (Goff, 2008). Thus, more and larger droplets of PL may migrate to the serum and sediment, increasing the values of $K_{PL}$. Indeed, droplets ranging in size from 30 to 60 and 40 to 90 $\mu$m were observed in the serum of LPL-ICM and HPL-ICM, respectively (Figure 3g and 3o). Subsequent hardening ($-50^\circ$C for 8 h) of the ice cream did not significantly change the $K_{PL}$ values for both samples ([4] in Figure 3). Hartel et al. (2017) estimated that 75% to 80% of the available water is frozen during hardening, concentrating the elements within the serum phase, such as dissolved sugars, lactose, milk proteins, and stabilizers. As water continues to freeze, the migration of PL from the coalesced and agglomerated emulsions into the serum becomes negligible due to mass transfer limitations within the frozen matrix (e.g., growth of ice crystals, increased viscosity, and low temperature).

**Effect of Phospholipids on Ice Cream Mixes**

**Viscosity.** Figure 4 presents the viscosity as a function of shear rate for mixes formulated with low- and high-PL content (~40 and 112 mg of PL·g$^{-1}$ of total fat, respectively), and Table 2 provides the yield stress ($\eta_o$), consistency index ($K$), and flow index ($n$). The viscosity for both mixes decreased with the shear rate, suggesting a shear thinning behavior. A shear thinning behavior has been reported in several ice creams, including regular (Sim et al., 2021), high protein (Daw and Hartel, 2015; Ranaweera et al., 2022), and low fat (Liu et al., 2018). Overall, the HPL-ICM resulted in a more viscous fluid than the LPL-ICM, regardless of the shear rate applied. An increment in viscosity of about 7–9-, 5–6-, and 3–4-fold was observed within the low (1–6 s$^{-1}$), medium (10–40 s$^{-1}$), and high (60–100 s$^{-1}$) spectrum of shear rate, respectively. Phospholipids are amphiphilic molecules that tend to form micellar or lamellar structures when dissolved, depending on the concentration, type of PL, and solvent (Arnold et al., 2013). However, experimental evidence of the PL structure within the ice cream is needed to support such a claim.

The Herschel-Buckley model was used to obtain the $\eta_o$, $K$, and $n$ for both mixes (Table 2). Colloidal suspensions are somewhat elastic and the $\eta_o$ represents the required shear rate for the suspension to flow. Interestingly, HPL-ICM displayed higher $\eta_o$ than the LPL-ICM (0.15 ± 0.09 and 0.016 ± 0.08 Pa, respec-
tively), indicating the minimum shear rate needed to break the structure of PL. Values of $K$ reflects the imparted viscosity (Table 2) due to the addition of PL, where HPL-ICM exhibited much higher $K$ than the LPL-ICM (15.93 ± 0.32 and 1.81 ± 0.09 Pa s⁻¹, respectively). An increase in the viscosity by adding PL can be a desirable attribute since a viscous mix limits the recrystallization, improves the smoothness, and reduces the melting rate (Amador et al., 2017; Hartel et al., 2017). A pseudoplasticity behavior ($n < 1.0$, Table 2) was observed for both samples, possibly by the disruption of the structured network as the shear is applied into the mix (Cavender and Kerr, 2020; Masselot et al., 2020).

**Particle Size Distribution and Zeta Potential.** The distribution of particles for LPL-ICM and HPL-ICM is given in Figure 5, where a relatively broad distribution of particles (~10 to 80 µm) characterized by 3 distinctive peaks was observed. The first peak spanned from 40 to 70 µm, corresponding to about 3% and 14% of the total particles for LPL-ICM and HPL-ICM, respectively. The second peak spanned from 6 to 30 and 4 to 20 µm for LPL-ICM and HPL-ICM, respectively, accounting for about 43% and 26% of the particles. The last peak accounted for about 53% and 59% of the total particles and spanned from 1 to 60 and 8 to 40 µm for LPL-ICM and HPL-ICM, respectively. A typical commercial mix may have a distribution of particles of 5 to 10 µm (Goff, 2022). Two-stage homogenization of the ice cream mixes would reduce the droplet size below 1 µm due to a combined effect of turbulence, cavitation, and shearing (Osorio-Arias et al., 2021).
Although the distribution of particles between LPL-ICM and HPL-ICM displayed similar broadness (~10 to 80 µm, Figure 5), the effect of PL on the viscosity was evident (Figure 4), where increments between 2- to 9-fold were observed. Several factors influence the development of viscosity in ICM, including composition, size of fat droplets, stabilizers, and temperature (Bolliger et al., 2000). As discussed earlier, ICM are colloidal suspensions with particles of opposite electrical charge to the solvent. A suitable method that may offer insights into the interaction between suspended proteins and fat globules is the electrokinetic potential or zeta potential. Michalski et al. (2002) evaluated mechanical damage of the MFGM during homogenization through zeta potential measurements, where native globules presented values of about −11 to −14 mV, and homogenized globules exhibited values of about −20 mV.

Interestingly, LPL-ICM presented values of zeta potential of −25.7 ± 6.3 mV, which correspond to the values of a homogenized MFGM. The casein micelles and whey proteins may surround the fat globules as opposed to a phospholipid membrane in the native form. On the contrary, HPL-ICM resulted in zeta potential values of −40.9 ± 6.7 mV, indicating a strong interaction between proteins, possibly casein micelles, and PL droplets within the surface of the fat globule.

**Protein Profile.** The protein distribution for LPL-ICM and HPL-ICM was evaluated through SDS-PAGE, (2) and (3) in Figure 6. Overall, both mixes displayed 4 distinctive bands at about 25, 20, 15, and 10 kDa, corresponding to β-CN, α-CN, β-LG, and α-LA, respectively. In addition to these bands, HPL-ICM presented protein bands associated with MFGM—xanthine oxidase, oxidase butyrophilin, and adipophilin at 100, 75, and 50 kDa, respectively.

**Effect of Phospholipids on Ice Cream Fat Destabilization, Overrun, and Hardness.** Table 3 presents the extent of fat destabilization, overrun, and hardness for the ice creams formulated with LPL and HPL. Ice creams formulated with LPL and HPL presented fat destabilization values of 77.18 ± 1.66% and 63.10 ± 2.10%, respectively. Similar values of fat destabilization (70%–80%) have been reported in 6%, 8%, and 12% fat (Adapa et al., 2000). In commercial ice creams, the extent of fat destabilization is quite variable from 5% to 84%, depending on the formulation, viscosity of the mix, type of ice cream, an emulsifier used, freezing protocols, overrun, and size of ice crystals (Amador et al., 2017).

Ice creams formulated with HPL imparted viscosity within the mix (Figure 4). Viscous mixes yield greater fat destabilization due to increased shear forces during freezing, inducing more collisions among the fat globules. The high concentration of PL (~112 mg per
g fat) may induce interactions between the suspended proteins and the fat globules that, combined with dynamic freezing conditions, result in partially coalesced fat globules.

Although both mixes contained a similar amount of fat (~11%, Table 1), samples formulated with LPL were higher than the HPL ice creams (14.11 ± 0.16% and 13.48 ± 0.67%, respectively, Table 3). The imparted viscosity of HPL negatively affects the foaming capacity, preventing air incorporation during freezing. The volume increase within the ice cream due to the incorporation of air is known as overrun, and it is related to several quality attributes, including the development of ice crystals, hardness, and melting behavior (Sofjan and Hartel, 2004).

The instrumental hardness, and maximum peak force, for the ice creams formulated with LPL and HPL were 180.51 ± 7.48 and 203.88 ± 8.47 N, respectively. Ice cream hardness is influenced by the size and number of ice crystals, mix viscosity, overrun, and fat destabilization (Amador et al., 2017). Values of ice cream hardness reported in the literature are quite variable because the hardness measurement is susceptible to the sample size, probe geometry, speed of the test, and penetration depth (Peleg, 2019).

Melting. Thermo-oscillatory rheology was used to evaluate the melting behavior of the formulated ice creams with LPL and HPL, where the storage (G') and loss (G'') moduli are expressed as a function of the temperature from −20°C to 10°C (Figure 7a). Overall, both ice creams displayed a sigmoidal behavior characterized by the distinctive presence of 2 plateaus and a linear segment located between the plateaus (Granger et al., 2004). Similar thermo-oscillatory curves have been reported in 5%, 10%, and 12% fat ice creams (Velásquez-Cock et al., 2019; Freire et al., 2020; Sim et al., 2021). Wildmoser et al. (2004) divided the thermo-oscillatory curves into 3 zones, according to the temperature range—zone I from 20°C to −10°C,
zone II from −10°C to 0°C, and zone III from 0°C to 10°C. In zone I, \(G'\) was slightly higher than \(G''\) for both samples, indicating a dominant solid behavior due to the presence of ice crystals. The HPL ice creams exhibited higher values of \(G'\) and \(G''\) than LPL ice cream, suggesting a more rigid structure due to the structuring ability of PL. These observations are coherent with the damping factor \(\tan(\delta) = G''/G'\), where LPL ice creams displayed higher values than HPL ice creams within the temperature range of −20°C to −10°C (Figure 7b). In the temperature range from −10°C to 0°C (zone II), the values of \(G'\) and \(G''\) linearly decreased with the temperature due to the melting of ice crystals, and the slope of the linear segment is associated with the speed of melting and the sensory impression of coldness (Wildmoser et al., 2004). During the melting of ice crystals, a predominant liquid behavior was observed (\(G' > G''\)) for both samples. Such behavior was more pronounced in LPL than the HPL ice creams, judging from the \(\tan(\delta)\) values (1.0 and 0.4, respectively, Figure 7b). The values of \(\tan(\delta)\) for the LPL varied from 0.32 to 102, whereas the \(\tan(\delta)\) values for HPL ice creams varied within a narrowed range (0.40–081, Figure 7b). The \(\tan(\delta)\) reached a maximum value at −3.63°C and −4.95°C for LPL and HPL ice creams, respectively. Upon further heating of the ice creams, the values of \(G'\) and \(G''\) remained unchanged from 0°C to 10°C since the ice crystals had already melted. After melting, the foam and the fat phase become the predominant behavior. In zone III, values of \(G'\) higher than \(G''\) indicated a sensory sensation of creaminess, higher for HPL than LPL ice creams.

Meltdown. The meltdown of ice creams formulated with LPL and HPL exhibited a sigmoidal curve with a distinctive lag, fast-melting, and plateau phase (Figure 8a), from which the onset, maximum rate, and maximum meltdown were obtained through nonlinear regression analysis. The ice creams formulated with HPL delayed the meltdown onset compared with the LPL ice creams (18.53 ± 0.57 and 14.83 ± 0.85 min, respectively). Such a delay may be attributed to the imparted viscosity of HPL-ICM, preventing the liquid drifts out of the fat network. Samples from the dripped portion were collected after the onset, maximum rate, and maximum meltdown for CLSM analysis (Figure 8b). Ice creams formulated with LPL exhibited larger droplets than HPL ice creams, which is consistent with the onset of meltdown. Overall, the meltdown behavior is affected by several factors, including formulation, the viscosity of the mix, overrun, fat destabilization, and number and size of ice crystals (Sofjan and Hartel, 2004; Amador et al., 2017).

After the onset of meltdown, melted ice crystals solubilized components within the serum phase and drained the fat phase. The maximum meltdown rate was higher in ice creams formulated with LPL than HPL ice creams (1.01 ± 0.05 and 0.71 ± 0.04% min⁻¹, respectively). The higher viscosity within the HPL-ICM yielded a slower rate of meltdown. The increase in temperature and gravitational force controls the maximum rate of meltdown. Microscope images at the maximum rate displayed larger droplets for ice creams formulated with LPL than HPL ice creams ([2] in Figure 8b), which is in agreement with the maximum rate values (1.01 ± 0.05 and 0.71 ± 0.04% min⁻¹, respectively). Subsequently, the meltdown values reached a plateau of 74.29 ± 2.26% and 66.18 ± 3.71% for LPL and HPL ice creams, respectively ([3] in Figure 8a). These values represent the maximum dripped portion of the ice cream, where the liquid is mainly made of melted ice crystals with soluble particles and residual emulsified fat (Sim et al., 2021). After reaching a plateau, the PL droplets for ice creams formulated with LPL displayed larger aggregates of droplets ([3] in Figure 8b).

Phospholipid droplets drain off the protein and fat phase during the meltdown, judging by the CLSM images at the onset, maximum rate, and maximum meltdown. The protein profile was analyzed before and after the meltdown of ice creams formulated with LPL and HPL ([4–6] in Figure 6). Before meltdown, LPL and HPL ice creams displayed strong bands that correspond to β-CN, α-CN, β-LG, and α-LA (10, 15, 20, and 25 kDa, respectively), whereas fading bands associated with the MFGM were observed. After the meltdown of the LPL and HPL ice creams ([3] in Figure 8a), the dripped liquid or serum showed the presence of protein bands associated with the membrane proteins (xanthine oxidase, oxidase butyrophilin, and adipophilin at 100, 75, and 50 kDa, respectively). This observation is coherent with the CLSM images after the maximum meltdown. Furthermore, the PL profile was analyzed through HPLC-CAD before and after the meltdown (Figure 9). Overall, 5 major classes of PL were identified, including PI, PS, PE, PC, and SM. Ice creams formulated with LPL showed a slight difference profile after the meltdown, where PC was not detected in the dripped portion ([2] in Figure 9). On the other hand, the PL profile of ice creams formulated with HPL resembles the profile reported in βS (Rathnakumar et al., 2021a). Phosphatidylcholine was not detected in HPL ice creams after the meltdown. During the meltdown, the temperature gradually increases, which induces changes in the concentration of minerals and stability of the fat globules. The melting behavior of ice creams is governed by several factors, including mix composition, particle size, mix viscosity, type processing, overrun,
fat destabilization, and the number of ice crystals (Sofjan and Hartel, 2004; Wu et al., 2019). Thus, the meltdown behavior of ice creams is highly variable in the literature, depending on the test conditions (amount of sample, size of wire mesh, temperature, and humidity).

Figure 7. Melting curve of ice creams formulated with different content of phospholipids. (a) Thermal variation of storage module ($G'$) and loss module ($G''$) and (b) thermal variation of loss tangent [tan(δ)]. LPL = low-phospholipid ice cream; HPL = high-phospholipid ice cream.
CONCLUSIONS

We studied the feasibility of replacing NFDM with βS during the manufacture of ice cream. Overall, ice cream formulated with βS resulted in a significantly higher content of PL, a 2.77-fold increment. The $K_{PL}$ and CLSM images indicated that the PL were predominantly found in the fat phase, and only a small amount was left in the serum and sediment. Subsequently, some migration of PL occurred from the emulsion into the

Figure 8. Meltdown of ice creams formulated with different content of phospholipids (a) and confocal laser scanning microscopy images of the phospholipid droplets from the dripped portion after maximum meltdown (b). LPL = low-phospholipid ice cream (IC); HPL = high-phospholipid ice cream. (1) Onset of meltdown, (2) maximum rate of meltdown, (3) maximum meltdown. The error bars correspond to the SD of 3 replicates.

Figure 9. Distribution of phospholipids before and after the meltdown. (1) Low-phospholipid ice cream before meltdown; (2) low-phospholipid ice cream after meltdown; (3) high-phospholipid ice cream before meltdown; and (4) high-phospholipid ice cream after the meltdown. PI = phosphatidylinositol; PS = phosphatidylserine; PE = phosphatidylethanolamine; PC = phosphatidylcholine; and SM = sphingomyelin.
serum after pasteurization and freezing. Higher PL content also resulted in an increment in viscosity of at least 4-fold, regardless of the shear rate. The ice creams formulated with HPL delayed the onset of meltdown due to the imparted viscosity of PL that prevents liquid from drifting out of the fat network. The outcomes of this investigation provide helpful information on the development of rational strategies to manufacture ice cream enriched with PL.

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Rathnakumar et al.: MONITORING PHOSPHOLIPIDS IN ICE CREAM MANUFACTURE


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