



Sunflower phospholipid fractions in ice cream: effects on emulsion stability, rheology, and microstructure

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Abstract

This study provides a quantitative evaluation of structure–function relationships in ice cream emulsions stabilized with phospholipid fractions derived from crude lecithin, a valuable byproduct after the degumming process in sunflower oil refining. Four purified fractions with varying phosphatidylcholine (PC) contents, crude lecithins and a commercial product, were incorporated into an optimized base formulation (1.04% salep, 0.48% phospholipids). Microstructural analysis revealed that Fraction 4 (highest PC content) produced the smallest and most uniform oil particles. These results correlated strongly with zeta potential and particle size (0.391 μm), confirming the role of PC enrichment in enhancing interfacial stability. While salep concentration primarily governed rheological properties (K ranged from 3.0 to 52.3 $\text{Pa}\cdot\text{s}^n$ and n from 0.43 to 0.72), phospholipid composition significantly affected zeta potential (–24.2 to –28.8 mV), particle size (0.427–0.547 μm), and overrun (22.7–46.2%). The findings highlight sunflower-derived phospholipids as sustainable, clean-label emulsifiers for ice cream.

Keywords Ice-cream · Lecithin · Phospholipid · Phosphatidylcholine · Emulsion · Salep

Introduction

Ice cream is a complex colloidal system composed primarily of fat, sugar, skim milk powder, flavoring, stabilizer, emulsifier, and water, and is widely consumed worldwide (Goff and Hartel, 2013a). Its structure includes dispersed fat globules, ice crystals, air bubbles, and a viscous serum phase, making it a multi-phase system with air/water and fat/water interfaces. The formulation and processing parameters directly influence its structural and sensory properties (Loffredi et al., 2021; Mohammed et al., 2022).

Fat, a key ingredient in ice cream formulation, influences textural and structural properties such as shape retention melt resistance and roughness after freezing (Genovese et al., 2022). Sugars not only provide sweetness but also lower the freezing point, helping ice cream remain soft at low temperatures. This prevents ice crystal growth, making it easier to shape, serve, and consume. Dairy cream

supports air cell formation and stability, which increases thermal resistance. Therefore, high-overrun ice cream melts more slowly than low-overrun varieties. Emulsifiers play a key role at the oil–water and air–water interfaces by reducing interfacial tension through their amphiphilic structure (Homayouni et al., 2018; Ludvigsen, 2015). However, their concentration must be balanced; excess disrupts air incorporation, while insufficient levels reduce stability. An optimal emulsifier ratio helps stabilize the matrix, ensuring cohesion between oil, air, and water phases critical to structure and quality (Chang et al., 2025). Stabilizers in ice cream formulations increase the viscosity of the unfrozen serum phase, affecting ice crystal formation and freezing rate. Salep, a natural polysaccharide-based stabilizer derived from wild orchid tubers (*Orchis* spp.), is used as one of the main stabilizers due to its high water-binding capacity and ability to improve texture in ice cream production (Bahramparvar and Tehrani, 2011; Genovese et al., 2022; Lal et al., 2006).

While semi-synthetic or synthetic emulsifiers like mono- and diglycerides (E471) are widely used, consumer demand for "clean-label" products has shifted focus toward natural alternatives, especially plant-derived phospholipids (Ivanova et al., 2024; Loffredi et al., 2021; Rinaldi et al., 2014). Crude lecithin contains neutral lipids (approximately 40%

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triglycerides), phospholipids (approximately 20–40%), free fatty acids, carbohydrates, glycolipids, color components and other minor oil components. Unlike soy lecithin, canola and sunflower lecithin are naturally non-GMO, allergen-free, and more acceptable in clean-label formulations (Bot et al., 2021; Deng et al., 2021). Phospholipids are amphiphilic molecules composed of a hydrophilic phosphate-linked head group and two hydrophobic fatty acid tails. These amphiphilic surfactants reduce the interfacial tension between two dense phases, such as oil and water. Naturally occurring phospholipids are found in lecithin, a byproduct of degumming, and the first step in the refining of vegetable oils. Crude lecithin is most commonly found in plant and animal sources such as soybeans, sunflower seeds, egg yolks, and rapeseed. The phospholipids in lecithin consist primarily of PC, phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidic acid (PA). PC and PE play a key functional role in food emulsions. PC, due to its cubic structure and strong surface activity, is particularly effective in stabilizing oil-in-water emulsions. Phospholipids are widely used in the formulation of chocolate, margarine, baked goods, baby food, and dairy desserts. According to the FDA, lecithin is a Generally Recognized as Safe (GRAS) additive with no maximum use limit in food applications, making it a valuable natural alternative to synthetic emulsifiers (Bot et al., 2021; Guiotto et al., 2015; Gutiérrez-Méndez et al., 2022).

Although lecithin is widely used as a natural emulsifier in the food industry, its functional performance is significantly influenced by its phospholipid composition. Changes in the ratios of PC, PE, and PI can significantly alter emulsion behavior, viscosity, and stability in complex food systems. While studies on the subject have investigated the use of crude lecithin in ice cream formulations, there is a significant lack of research comparing the functional effects of specific phospholipid fractions. Most existing studies treat lecithin as a single additive without considering the potential differences that arise with fractionation and phospholipid enrichment (Rinaldi et al., 2014). Therefore, the comparative evaluation of lecithin fractions with different phospholipid compositions on the structural properties of ice cream represents a novel approach.

This study aimed to investigate the structure–function relationship in ice cream emulsions stabilized with sunflower phospholipid fractions of varying PC content. Crude sunflower lecithin obtained from the degumming step of oil refining was fractionated to produce clean-label, functional emulsifiers. A CCD was used to optimize the salep and phospholipid concentrations, enabling the development of stable ice cream formulations. The selected emulsions were evaluated using a range of parameters, including zeta potential and particle size analysis, quantitative microstructural imaging (droplet number, size distribution, and homogeneity), rheological profiling, overrun, thermal characterization

(DSC), and color. The findings provide a quantitative link between emulsion stability and microstructure in ice cream with varying PC content. Moreover, this research introduces an innovative route for upcycling refining byproducts into high-value, functional food ingredients, supporting both clean-label formulation and circular economy principles.

Materials and methods

Materials

Ice cream samples were prepared using beet sugar (Pakmaya, Türkiye), milk cream (18% milk fat, Süttaş, Türkiye), skim milk powder (contains 35% protein and 52% carbohydrate, Alfamol, Başakşehir/ İstanbul), water, and salep (*Orchis mascula*, Aktarix, Burdur/Türkiye) as a natural stabilizer. All ingredients were procured from local suppliers in Türkiye. Crude lecithin, recovered as a byproduct of the water degumming step during sunflower oil refining, was supplied by Reka Oil Company (Tekirdağ, Turkey). Commercial ice cream was supplied from the local market as "Maraş-style" ice cream. All reagents and solvents used in analysis were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany).

Production and analysis of phospholipid fractions

Four phospholipid fractions with varying PC-PE-PI contents were obtained from crude sunflower lecithin using a standardized purification protocol based on food-grade solvent fractionation, as described in the literature (Cabezas et al., 2013; Nieuwenhuyzen and Tomás, 2008; Zhang et al., 2003). For phospholipid analysis, a calibration curve was initially obtained using the PC-PE-PI standard mixture at five different concentrations, as described by Yu et al. An Agilent 1260 Infinity II HPLC instrument, equipped with an Agilent 1260 Infinity II Diode Array Detector WR, an Agilent 1260 Quat Pump VL, and an Agilent 1260 Vialsampler were used. All analyses were performed using an SVEA C:18 Gold (250×4.6 mm, 5 µm) (A585V1) column. The entire phospholipid analysis process used the method of Yu et al. (Yu et al., 2022).

Production of ice creams

The ice cream formula used in this study was adapted from the formulation proposed by (Goff and Hartel, 2013b). According to this modified formula, the ice cream mix consisted of 10% cream, 15% sucrose, and 11% skim

milk powder, with the remainder consisting of water. The amounts of phospholipid fractions (0–0.56%) and salep (0.75–1.75%) were varied according to the experimental design (Table 2). To ensure consistency in the total mass of the formulation, the water content was adjusted according to the levels of these two variables. The ice cream production procedure was adapted from the method described by (Arslan et al., 2023), with minor modifications. All ingredients were mixed under controlled conditions to ensure homogeneity before freezing. To prepare the ice cream mix, water was first heated to 50 °C. Once the temperature reached 60 °C, cream, phospholipid-based emulsifier, skim milk powder, sugar, and salep were added sequentially with continuous stirring. The mixture was then heated to 80 °C and pasteurized by holding at this temperature for 10 min with constant agitation to ensure homogeneity (Thermomix, Vorwerk, Germany). After pasteurization, the mixture was allowed to cool to ambient conditions and then aged at 4 °C for 18–24 h. Following aging, the mixture was transferred to an ice cream machine (DeLonghi IL Gelataio ICK5000, Treviso, Italy) and frozen at -10 °C for 30 min. The resulting ice cream samples were stored in a deep freezer at -18 °C until analysis. Each sample container was appropriately labeled with a unique identification code and the production date. All analytical measurements were performed the day following production.

Experimental design

The experimental design was generated using Design Expert version 7.0.0 software (State-Ease Inc., USA). One-way analysis of variance (ANOVA) and Tukey's test

Table 1 Contents of PC, PE, and PI in Phospholipid Fractions and Crude Lecithins (%)

	Phospholipid rate (%)	PC (%)	PE (%)	PI (%)
Phospholipid Fraction 1	42.3	23.8	10.5	8
Phospholipid Fraction 2	61.1	25.5	20.8	14.7
Phospholipid Fraction 3	99	57.6	35.8	6.5
Phospholipid Fraction 4	99	62.3	32.7	4.9
Crude sunflower lecithin	28	14.3	7.6	6.1
Crude soybean lecithin	39.8	21.8	10.8	7.2

Fractions were produced from crude sunflower lecithin

PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol

were performed using the SPSS 16.0 statistical package for Windows (SPSS Inc., USA). Response Surface Methodology with rotatable CCD was generated using the ratios of the two components of ice cream, salep and phospholipids. In this experimental design, phospholipid fraction 1, with the properties specified in Table 1, was used. The experimental points of the generated experimental design are shown in Table 2. Salep concentration and phospholipid concentration (0–0.56%) were included as variables in the optimization design. Phospholipid type, however, was not included directly in the design; instead, once the optimal base formulation was established (1.04% salep and 0.48% phospholipid), different phospholipid fractions with varying PC/PE/PI compositions were tested under identical conditions. This two-step approach allowed the independent evaluation of phospholipid composition without confounding effects from salep concentration.

Rheological measurements

The rheological properties of ice cream mixes were evaluated using a stress- and temperature-controlled rotational rheometer (MCR 302, Anton Paar, Austria) equipped with a parallel plate geometry (PP50, 50 mm diameter) and a gap size of 0.5 mm. All measurements were performed at a constant temperature of 10 °C. Steady shear tests were conducted over a shear rate range of 0.1 to 100 s⁻¹ to obtain shear stress and apparent viscosity curves. The flow behavior of each sample was modeled using the Power Law model, in Eq. 1:

$$\tau = K\gamma^n \quad (1)$$

where τ is the shear stress (Pa), γ is the shear rate (s⁻¹), K is the consistency coefficient (Pa·sⁿ), and n is the flow behavior index (dimensionless). Nonlinear regression analysis was applied to calculate K , n , and the coefficient of determination (R^2) for each sample. All measurements were performed three times to ensure repeatability (Chen et al., 2019; Farahmandfar et al., 2019).

Zeta potential value and particle size

Samples were diluted 1:500 in 5 mM phosphate buffer prior to zeta potential measurement using Malvern Mastersizer Nano ZS90 (Malvern instrument Co. Ltd, UK) at 25 °C, and measurements were performed triplicate. Droplet size distribution was measured by a Mastersizer 2000 (Malvern instrument Co. LTD, UK). Each 1 mL emulsion was diluted one thousand times with distilled water (Arslan et al., 2023).

Table 2 Experimental points of CCD experimental design

Run	Salep (%)	Phospholipid (%)	Cream (%)	Sugar (%)	Skimmed milk powder (%)	Water (%)
E1	0.75	0.48	10	15	11	62.77
E2	1.25	0	10	15	11	62.75
E3	1.25	0.56	10	15	11	62.19
E4	1.75	0.48	10	15	11	61.77
E5	1.96	0.28	10	15	11	61.76
E6	0.54	0.28	10	15	11	63.18
E7	1.75	0.08	10	15	11	62.17
E8	0.75	0.08	10	15	11	63.17
E9	1.25	0.28	10	15	11	62.47

Phospholipid fraction 1 used

Thermal properties

To investigate the thermal behavior of ice cream samples, a differential scanning calorimeter (DSC) device (STA44gf3, Netzsch, Germany) operating at atmospheric pressure was used. Approximately 15 mg of each sample was placed in pre-weighed aluminum sample cups and then hermetically sealed with a Tzero-type Quick Press cup crimper. Analyses were carried out under nitrogen gas at a flow rate of 20 mL/min to provide an inert environment. Measurements were carried out between $-20\text{ }^{\circ}\text{C}$ and $+50\text{ }^{\circ}\text{C}$ at a constant heating rate of $5\text{ }^{\circ}\text{C}$ per minute. An empty aluminum cup was used as a reference. Melting enthalpy (ΔH), peak temperature (T_{peak}), and end-melting temperature (T_{end}) were determined from thermograms (Arslan et al., 2023).

Color analysis

Color measurements of the ice cream samples were conducted using a CR-400 Chroma Meter (Konica Minolta, Tokyo, Japan), which was calibrated prior to analysis. Each sample was measured at three different points, and the results were averaged. The color parameters included L (lightness, ranging from 0 = black to 100 = white), a (positive values indicating redness and negative values indicating greenness), and b* (positive values indicating yellowness and negative values indicating blueness) (Azmoon et al., 2021; Kumar et al., 2011).

Overrun analysis

To assess the volume reduction of ice cream samples, each sample was carefully transferred to a pre-tared graduated cylinder, ensuring the entire target volume was filled without any voids. This process was performed in the same manner for all samples, and their weights were recorded using a precision

balance. The samples were then allowed to thaw at ambient temperature. The melted samples were then poured into the graduated cylinder, and the weights were measured again using the same precision balance. The volume reduction was calculated using the formula presented in Eq. 2 (Kumar et al., 2011).

$$\text{Overrun (\%)} = (V1 - V2)/V1 * 100 \quad (2)$$

Texture profile analysis (TPA)

The textural parameters (hardness, springiness, cohesiveness, chewiness) of ice cream (stored at $-18\text{ }^{\circ}\text{C}$) by the texture analyzer (SMS TA.XT2 Plus, UK) with a 36 mm diameter cylindrical compression probe and a 100 kg load cell. During analysis, crumbs were subjected to two compression cycles with a $5\text{ mm}\cdot\text{s}^{-1}$ delay time. TPA parameters were 50% compression and 5.0 mm/s test speed. Triplicate measurements were performed (Olçay and Özülkü, 2025).

Microstructure analysis

The microstructure of the ice cream samples was examined using a polarized light microscope (PLM, Zeiss Axio Imager A2, Carl Zeiss, Germany). After whipping, the mixture was applied to a depression slide covered with a coverslip and observed under $10\times$ objectives at $+4\text{ }^{\circ}\text{C}$ (Erem et al., 2023). The microscopic imaging combined with threshold-based segmentation was performed using ImageJ software (Washington, USA). The imaging was conducted using a standard light microscope without staining.

Result and discussion

Optimization of parameters

Rheological properties

According to the results of ANOVA, salep concentration had a statistically significant effect on both the consistency coefficient (K) and the flow behavior index (n) of the ice cream mixes ($p < 0.05$), while phospholipid content did not exhibit a statistically significant influence ($p > 0.05$), (for K ; $R^2 = 0.8352$, for n ; $R^2 = 0.7021$). The K ranged from 3 to 52.3 ($\text{Pa} \cdot \text{s}^n$) and the n ranged from 0.43 to 0.72 (Table 3). These findings indicate that the rheological behavior of the ice cream mix is mainly controlled by salep, which is a hydrocolloid with high polysaccharide content known to increase the viscosity of the water phase and improve structural resistance during flow. As the salep ratio increased, the K value also increased, showing greater resistance to shear. In all samples, the n values were found to be less than 1, indicating that the viscosity of the mix decreased as the shear rate increased, which is characteristic of pseudoplastic (shear-thinning) flow behavior commonly observed in food systems containing stabilizers like salep. Furthermore, the changes in n values depending on salep level suggest that salep not only thickens the mix but also affects how the viscosity responds to mechanical force during flow. On the other hand, phospholipid fractions added at levels up to 0.56% did not significantly affect either K or n . This could be because phospholipids primarily function at the interfaces between fat and water or air and water, playing a more pronounced role in emulsion stability and air incorporation, rather than in bulk viscosity or flow resistance. Their relatively low usage level compared to salep may also explain this limited effect on shear behavior (Fig. 1).

The rheological results obtained in this study are consistent with previous findings reported by Arslan et al., who evaluated the effect of different salep types on ice cream mixes. In those studies, higher salep content and glucomannan-rich varieties were associated with a significant increase in the consistency coefficient (K) and a decrease in the flow behavior index (n), indicating stronger resistance to shear and enhanced pseudoplasticity (Arslan et al., 2023). According to Parvar et al., ice cream samples prepared with κ -carrageenan exhibited consistency coefficients (K) ranging from 19.51 to 20.41 ($\text{Pa} \cdot \text{s}^n$) and flow behavior indices (n) below 1, reflecting non-Newtonian shear thinning behavior (Bahram Parvar et al., 2013). Similarly, in the present work, increasing the salep level from 0.75% to 1.75% led to a substantial increase in K values (from 3.00 to 52.36 $\text{Pa} \cdot \text{s}^n$) and a corresponding decrease in n values (from 0.72 to 0.43), confirming that salep plays a dominant role in controlling the flow properties of the ice cream mix. Based on the data presented by Anpar et al., the n and K values were obtained between 0.38–0.68 and 0.24–11.52 ($\text{Pa} \cdot \text{s}^n$), respectively, with the use of stabilizers at different ratios in ice cream mixes, and the changes in K and n values are also consistent with the present study (Azari-Anpar et al., 2017). These findings confirm that increasing stabilizer concentration increases intrinsic viscosity while simultaneously decreasing the flow behavior index (n), thus enhancing shear-thinning behavior.

It is noteworthy that in previous studies, mono- and diglycerides (MDGs) were used at levels of approximately 0.3–2.0% in combination with low stabilizer concentrations (e.g., a mixture of 0.2% carboxymethylcellulose and tare gum), and significant changes in the consistency coefficient (K) and the flow behavior index (n) were observed (Lofredi et al., 2021). In the present study, although phospholipids were added up to 0.56%, their effects on rheological parameters were not statistically significant. This is likely due to the relatively high concentration of salep used in the formulations (up to 1.75%), which dominated the rheological

Table 3 Response Values Obtained from the CCD Design

Run	K ($\text{Pa} \cdot \text{s}^n$)	n	R^2	Overrun (%)	Zeta Potential (mV)	Particle Size (μm)
E1	9.838	0.5598	0.9568	28.15	-28.0	0.435
E2	20.095	0.5071	0.9435	46.22	-24.2	0.504
E3	19.236	0.5482	0.9718	23.35	-28.8	0.427
E4	29.668	0.5057	0.9128	25.66	-26.0	0.452
E5	52.356	0.4302	0.9517	24.07	-25.1	0.501
E6	3.004	0.7208	0.9094	22.75	-24.9	0.512
E7	34.568	0.5101	0.9305	36.43	-26.5	0.496
E8	6.061	0.6501	0.9317	33.92	-24.3	0.547
E9	6.768	0.6554	0.8846	40.82	-27.6	0.478

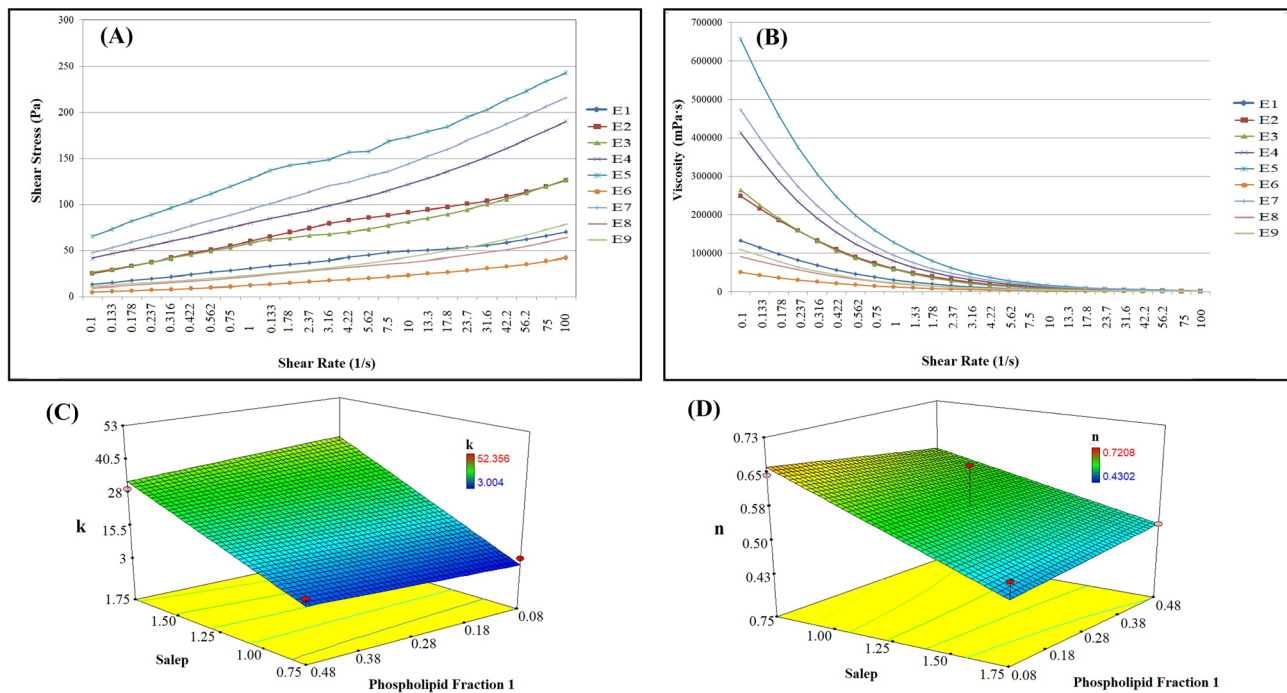


Fig. 1 Flow behaviour and model responses of ice-cream mixes formulated with varying salep and phospholipid fraction 1. **A:** Shear stress versus shear rate curves, **B:** Apparent viscosity versus shear

rate curves, **C:** Response-surface plot showing the effect of salep and phospholipid fraction level on the consistency coefficient (k , Pa·s ^{n}), **D:** Response surface plot for the flow-behaviour index (n)

response, as well as to the distinct functional mechanisms of phospholipids compared to MDGs.

Zeta potential value, particle size and overrun

According to ANOVA results, phospholipid content had a statistically significant effect on both zeta potential and particle size of ice cream mixes ($p < 0.05$), while salep concentration had no significant effect on any of these parameters ($p > 0.05$) (for zeta potential: $R^2 = 0.9216$; for particle size: $R^2 = 0.9721$). The zeta potential ranged from -24.2 to -28.8 mV, and the particle size ranged from 0.427 to 0.547 μm (Table 3). These findings indicate that the emulsion properties of the ice cream matrix are primarily influenced by the emulsifier system, particularly the phospholipid fraction, rather than the salep stabilizer. As phospholipid content increased, zeta potential values became more negative, indicating increased electrostatic repulsion between dispersed particles and, consequently, higher emulsion stability. Additionally, a decrease in average particle size was observed with increasing phospholipid content

(Fig. 2). This suggests that phospholipids more effectively cover the oil–water interface, resulting in smaller and more stable emulsion droplets. This behavior is consistent with the expected functionality of natural emulsifiers in complex food systems such as ice cream, where air incorporation and the efficiency of droplet stabilization directly affect product quality (Nyuydze and Martínez-Monteaudo, 2021).

The amount of overrun in ice cream is important for its texture and melting quality in the mouth. According to the ANOVA results, phospholipid concentration had a statistically significant effect on the overrun value of ice cream samples ($p < 0.05$), while salep content was not found to be statistically significant ($p > 0.05$), ($R^2 = 0.9321$). The overrun ranged from 22.75 to 46.22% (Table 3). As seen in Fig. 2, overrun value decreased as the amount of phospholipids increased. The highest overrun value was achieved with the least phospholipids, while the lowest overrun value was achieved with high concentrations of phospholipids. This inverse relationship can be attributed to the functional behavior of phospholipids at the air–water and oil–water interfaces. At high levels, phospholipids can over-stabilize the oil globule surface and prevent partial coalescence

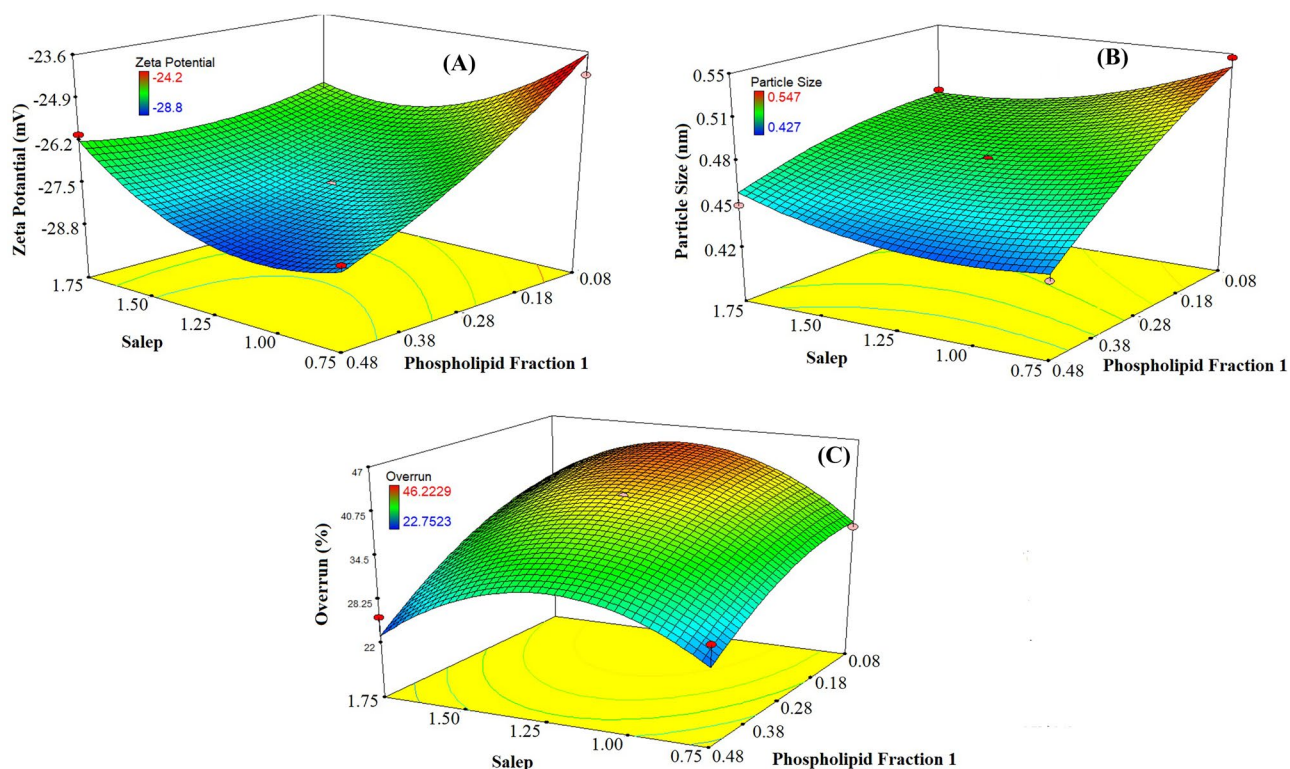


Fig. 2 Zeta potential (A), particle size (B) and overrun (C) model responses of ice-cream mixes formulated with varying salep and phospholipid fraction 1

necessary for effective air incorporation during freezing, consequently reducing the amount of air trapped in the mixture. In other words, phospholipids increase emulsion stability, while high interfacial activities can disrupt the foaming and aeration processes required for high overrun, resulting in a denser product. Similar trends have been reported in studies evaluating the effects of natural emulsifiers on ice cream structure; in these studies, it was observed that increasing surfactants decreased air incorporation due to changing interfacial behavior (Baer et al., 1997; Rinaldi et al., 2014).

Optimization of formulation parameters

The optimization process resulted in a formulation that minimized both particle size and zeta potential (improved emulsion stability) while maintaining the consistency coefficient (K), flow behavior index (n), and overrun values within acceptable limits. The numerical optimization yielded an optimal formulation of 1.04% salep and 0.48% phospholipid fraction 1, with K: 14.68 Pa·sⁿ, n: 0.583, overrun: 31.20%, zeta potential: -28.58 mV, particle size: 0.436 µm, and an overall desirability value of 0.940.

Ice cream production with optimized formulation: comparison of different phospholipid fractions and commercial products

In the first phase of this study, the effects of varying salep and phospholipid concentrations on the rheological and emulsion-related properties of ice cream mixes formulated with Phospholipid Fraction 1 were investigated, and an optimal formula was obtained. In the second phase, comparative analyses of the properties of phospholipid fractions with different PC, PE, and PI contents were conducted based on this optimized formulation. Additionally, ice cream was produced under similar conditions using industrially available crude sunflower and crude soy lecithins. A commercially available ice cream sample containing salep was compared with all other phospholipid sources.

All samples were prepared under the same conditions using the optimized base mixture (1.04% salep and 0.48% phospholipid) and subjected to comparative analysis for rheological properties, zeta potential, particle size distribution, overrun, DSC-based thermal behavior, color parameters and microscopic imaging. This two-stage design allowed for a controlled evaluation of how PC, PE, and PI fractions in the phospholipid composition affect ice cream stability

and quality characteristics. It also allowed comparison with commercially used crude lecithin and ice cream produced commercially with MDG and salep.

Rheological properties

The rheological properties of the phospholipid fractions, crude sunflower lecithin, crude soy lecithin, and a commercial ice cream sample, were evaluated in terms of consistency index (K) and flow behavior index (n). No consistent trend in K values was observed among the phospholipid fractions. This indicates that changes in phospholipid compositions, including phosphatidylcholine (PC) content, did not lead to a systematic increase or decrease in viscosity. Crude soy lecithin showed a more pseudoplastic behavior, exhibiting the highest K value (26.618 Pa·s ^{n}) and the lowest n value (0.5033). The first step of the study revealed that phospholipid content alone did not significantly affect the rheological parameters (K and n) at a statistically significant level. In contrast, the commercial ice cream sample showed a Newtonian-like behavior with a significantly lower consistency index (0.1139 Pa·s ^{n}) and a higher flow index (1.4343), reflecting its fluid-like nature (Table 4-Additional file).

Zeta potential value, particle size and overrun

Zeta potential

In general, a highly negative zeta potential enhances emulsion stability by providing electrostatic repulsion that prevents droplet coalescence. In the present study, zeta potential values of ice cream samples ranged from −21.4 mV to −34.1 mV (Table 4-Additional file). Among the phospholipid fractions, Fraction 3 exhibited the most negative zeta potential (−23.7 mV), whereas Fraction 4, despite having the highest PC content, showed a lower zeta potential (−21.7 mV). This result may seem counterintuitive at first, but it is consistent with the physicochemical nature of PC. Unlike phospholipids such as PE or PI, which contribute more strongly to surface charge, PC is a zwitterionic molecule with a neutral head group, and therefore contributes less to electrostatic repulsion. However, PC stabilizes emulsions through steric interactions, thanks to its large, hydrated choline head group that creates a physical barrier around fat droplets. This steric hindrance limits droplet coalescence even when zeta potential is relatively low. Literature studies have reported cases where nanosuspensions with zeta potentials below 20 mV were physically stable. This could be explained by the addition of non-ionic surfactants such as PC and the resulting steric effects (Cortés et al., 2021).

Steric effects are a type of repulsive force that occurs when bulky molecular groups on the surface of a particle physically prevent other particles from approaching the surface (Gotchev et al., 2011). This effect prevents direct contact between particles, preventing undesirable phenomena such as coalescence and sedimentation, thereby increasing system stability. In such systems, the structural barrier formed by surface-active phospholipids may reduce the need for strong electrostatic repulsion, thus lowering the zeta potential while maintaining emulsion integrity. The smaller, well-dispersed droplets are less likely to collide or aggregate, supporting long-term stability through steric hindrance rather than charge-based mechanisms. PC, being zwitterionic, contributes primarily to steric stabilization with little effect on zeta potential, whereas PI, bearing a net negative charge, enhances electrostatic repulsion between droplets, resulting in more negative zeta values (Bot et al., 2021; Gutiérrez-Méndez et al., 2022).

In contrast, the commercial ice cream sample showed the most negative zeta potential (−34.1 mV), likely due to the presence of highly effective emulsifiers like mono- and diglycerides (MDGs) and stabilizers that increase interfacial charge and dispersion stability through both electrostatic and steric mechanisms. Overall, while zeta potential values alone may not fully capture the stabilization effect of certain phospholipid types, the results suggest that steric stabilization from PC-rich fractions like Fraction 4 plays a crucial role in improving emulsion homogeneity, as also supported by microscopic observations.

Particle size

When particle size was examined across the seven samples, a significant decreasing trend was observed among the phospholipid fractions, from Fraction 1 (0.568 μ m) to Fraction 4 (0.391 μ m). This gradual reduction in particle size correlates with increasing PC content, suggesting that higher PC levels contribute to the formation of smaller and more uniformly distributed fat droplets. This is likely due to the effective interfacial activity and emulsifying capacity of PC, which enhances droplet dispersion and limits coalescence. Crude sunflower and soy lecithins exhibited slightly larger particle sizes (0.624 μ m and 0.635 μ m, respectively), probably due to their more complex and heterogeneous compositions. In contrast, the commercial sample showed the smallest particle size (0.523 μ m); this may be attributed to the use of additional processing techniques such as high-shear homogenization and the presence of synthetic emulsifiers. These results, together with the zeta potential and microscope images data, indicate that phospholipid composition (especially PC level) plays an important role in both colloidal stability and particle size distribution (Table 4-Additional file). The information that the use of lecithin reduces particle size distribution

due to phospholipid incorporation is consistent with the literature (Nyuydze and Martínez-Monteagudo, 2021).

Overrun

Overrun values of ice cream samples were evaluated in seven samples to assess air incorporation and the resulting foam structure stability. Overrun values were relatively similar among the phospholipid fractions, ranging from 26.51 to 29.26%, indicating that increasing PC content did not lead to a clear or consistent effect on air incorporation. Fraction 2 achieved the highest overrun value among the fractions, but this value did not provide a significant functional advantage. As noted in the first step of the study, increased phospholipid activity in ice cream leads to a statistically significant decrease in overrun. Crude sunflower and soy lecithins showed slightly higher values (28.55 and 30.71%), likely promoting air entrapment due to their relatively low phospholipid content. The commercial ice cream sample, however, exhibited the lowest overrun value (10%) (Table 4-Additional file). This result is consistent with the characteristics of traditional “Maraş-style” ice cream, which is typically dense and chewy due to its slow-churning and pounding technique both of which limit air incorporation. Such products are intentionally formulated with low overrun. Although MDG is generally associated with high overrun values in industrial ice cream formulations, the low overrun observed here may reflect different stabilizer-to-emulsifier ratios, process conditions, or intentional formulation design (e.g., denser product). In the study conducted by Rinaldi et al., the highest overrun value was achieved with the use of MDG (Rinaldi et al., 2014). Similarly, a decrease in overrun was reported in low-fat ice creams as a result of the use of soy lecithin as an emulsifier compared to MDG (Baer et al., 1997).

Thermal properties

Differential scanning calorimetry (DSC) analysis was performed to study the thermal transitions of fractions with various phospholipid compositions, crude lecithins, and commercial ice cream samples, and to understand the amount of free and bound water in the matrix. Across the phospholipid fractions, a gradual decrease in melting enthalpy (ΔH) was observed from Fraction 1 to Fraction 4 (from 182.6 J/g to 147.4 J/g) and a slight decrease in peak melting temperature (T_{peak}) from -4.05 °C to -6.43 °C (Table 4-Additional file). This trend shows a parallel decrease with increasing PC content. This may indicate that due to the amphiphilic nature of PC, its water binding capacity increases, allowing it to interact with free water molecules and reduce the amount of unbound free water available for crystallization. A

decrease in ΔH generally indicates a decrease in the amount of freezable water and reflects greater water immobilization within the system (Kasapoglu et al., 2023). Similar effects were reported by Rinaldi et al., who observed increased melting resistance in ice creams containing soy phospholipids, which was attributed to higher levels of bound water (Rinaldi et al., 2014). In this context, the observed shifts in T_{peak} and T_{end} values further support the hypothesis that phospholipids, particularly those rich in PC, may delay melting by structuring the water phase.

Color measurement

Color measurements of ice cream samples were evaluated using CIE Lab* parameters to assess the visual impact of different phospholipid sources. Across the phospholipid fractions, a significant increase in brightness (L^*) was observed from Fraction 1 (43.65) to Fraction 2 (58.91); Fractions 3 and 4 showed similarly high values (55.78 and 58.39, respectively). This trend suggests that higher PC content may contribute to a lighter appearance due to potentially improved emulsion stability and more uniform distribution of the components. All samples showed negative a^* values, indicating a slightly greenish tint; more negative values were present in fractions with higher PC levels. The b^* values, representing yellowness, were also higher in these fractions (approximately 7.3–7.5) compared to crude lecithins (approximately 5.2–5.4). Crude sunflower and soy lecithins had similar color values to Fraction 1, with lower L^* and b^* values, indicating a darker, less yellow hue. The commercial sample exhibited a moderate lightness ($L^* = 49.83$) and the lowest b^* value (3.02), likely due to formulation differences and the presence of whitening agents or stabilizers. Overall, the results indicate that phospholipid composition, particularly PC content, has a measurable effect on the visual properties of the ice cream matrix (Arslan et al., 2023; Rinaldi et al., 2014).

Microscopic evaluation of emulsion structure

Microscopic imaging under $10\times$ and $20\times$ magnification, combined with threshold segmentation using ImageJ, was conducted to qualitatively evaluate the emulsion structures of ice cream samples prepared with different phospholipid fractions and crude lecithins. Red regions in the processed images indicate oil-rich domains, while black-ringed formations correspond to encapsulated fat droplets surrounded by phospholipids. The observations provide visual confirmation of the emulsifying performance of different phospholipid fractions and crude lecithins used in ice cream formulations.

Among the tested formulations, Fraction 4 exhibited the most uniform distribution of small and well-dispersed oil droplets, with minimal aggregation and coalescence. This fraction had the highest particle count ($n=540$) combined with a relatively small average droplet area ($1215 \pm 443 \mu\text{m}^2$), indicating a highly effective emulsion structure with low variability. This sample showed widespread red regions with few large black-ringed structures, suggesting effective surface coverage and interfacial stabilization likely due to its high PC content. PC, known for its strong surface activity and ability to align at the oil–water interface, appears to contribute significantly to the stabilization of the emulsion microstructure.

In contrast, despite its similarly high phospholipid content, Fraction 3 exhibited a more heterogeneous structure with an increased number of larger oil droplets. Fractions 2 and 3, characterized by lower droplet counts and higher standard deviations, likely contained coalesced fat droplets, implying limited emulsification capacity and reduced stability. Fraction 1, although presenting the highest droplet count ($n=708$), displayed a considerably lower average droplet area ($474 \pm 2108 \mu\text{m}^2$), reflecting high variability and the presence of droplets with diverse sizes. This heterogeneity suggests an increased likelihood of droplet coalescence, which compromises overall emulsion homogeneity.

The samples prepared with crude sunflower and soybean lecithins exhibited pronounced structural irregularities. Despite their moderate droplet counts, these samples showed the highest standard deviations, resulting in non-uniform droplet sizes and poor emulsification performance. These images revealed large, irregular oil droplet formations and patchy red regions, implying poor dispersion and encapsulation. The variability in droplet size and spatial distribution in these samples may result from their lower phospholipid purity and more heterogeneous phospholipid profiles, which limit their interfacial activity and emulsifying capacity (Fig. 3). Threshold-processed images highlight

fat-rich domains in red, representing dispersed fat globules, whereas black-ringed formations correspond to larger aggregated droplets. This visualization provides a clearer comparison of droplet size distribution and degree of aggregation among the samples. Similarly, Rathnakumar et al., demonstrated with Confocal Laser Scanning Microscopy images that ice cream mixes with high phospholipid content contained smaller and densely distributed phospholipid droplets (Rathnakumar et al., 2023).

Overall, the trend observed in the microscopic images corroborates the zeta potential and particle size measurements. Higher PC content was associated with smaller droplet size, enhanced dispersion, and improved structural uniformity. These findings confirm that phospholipid fractionation and enrichment, particularly in PC, play a critical role in promoting fine droplet distribution and improving emulsion stability in structured food systems such as ice cream.

Texture profile analysis

Texture profile analysis showed that changes in PC content did not have a consistent or clear effect on hardness, adhesiveness, springiness, or cohesiveness. Although the highest hardness value was observed in the sample with the highest PC content, the overall trend across samples was uneven; indicating that PC level alone was not a determining factor for textural properties. Similarly, elasticity and cohesion fluctuated without a clear pattern, while cohesiveness remained negative for all samples. These findings, in agreement with the rheological data, highlight the influence of other formulation factors and ingredient interactions, indicating that PC content alone does not play a decisive role in determining the structural or flow properties of ice cream. When ice cream hardness values are compared with the literature, it is seen that the results are quite variable. This is

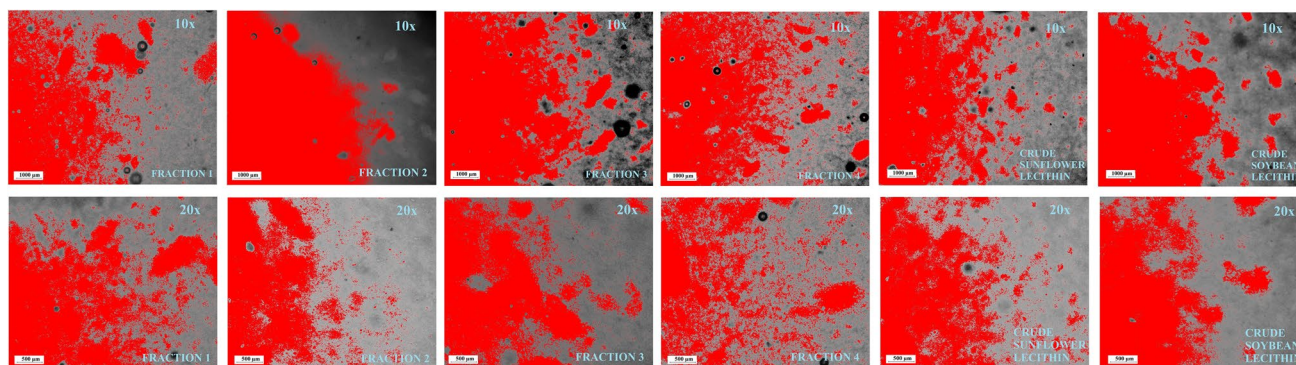


Fig. 3 Threshold-processed microscopic images (10× and 20×) of ice cream emulsions stabilized by different phospholipid fractions and crude lecithins

because the hardness measurement can vary depending on the sample size, probe geometry, test speed and penetration depth (Table 5-Additional file).

Comparative evaluation of phospholipid fractions

This study highlights the potential of sunflower-derived phospholipid fractions, particularly those enriched in phosphatidylcholine (PC), to enhance emulsion stability in ice cream formulations. The results demonstrated that both salep concentration and phospholipid composition significantly influenced the rheological and physical characteristics of the mixes. All formulations exhibited pseudoplastic (shear-thinning) flow behavior, confirming the typical response of systems containing hydrocolloid stabilizers. Salep concentration mainly affected viscosity parameters (K and n), whereas the type of phospholipid fraction strongly influenced emulsion stability and structural attributes. Among the tested fractions, Fraction 4 (with the highest PC content) provided the smallest particle size ($0.39\ \mu\text{m}$) and relatively stable zeta potential values ($-21.7\ \text{mV}$), indicating strong interfacial stabilization. Microscopy results were consistent with these data, showing more homogeneously distributed droplets compared with crude lecithins, where large and irregular domains were evident. Threshold-processed images confirmed that red regions represented fat-rich domains, while black-ringed areas corresponded to aggregated droplets.

In terms of overrun, crude lecithins showed slightly higher values (28–31%) than Fraction 4 (26.8%), suggesting that while PC enrichment improved droplet stabilization, it did not directly translate into increased air incorporation. Nevertheless, the commercial product displayed the lowest overrun (10.4%), emphasizing that all lecithin-containing samples performed markedly better in aeration compared with synthetic alternatives. Thermal behavior was also affected by phospholipid type. Fraction 4 showed a lower peak melting temperature compared with crude lecithins, which may be associated with altered crystallization dynamics. Textural measurements supported these findings, as Fraction 4 yielded the highest hardness values, indicating a more compact and rigid structure. Such characteristics may be advantageous for improving melting resistance and shape retention during storage and consumption.

Overall, the use of fractionated phospholipids, especially Fraction 4, resulted in improved microstructural uniformity and enhanced colloidal stability compared with crude lecithins and commercial samples. Although no single natural emulsifier matched the multifunctionality of monoglycerides, these findings suggest that targeted phospholipid enrichment can provide a promising clean-label alternative

for stabilizing ice cream matrices. From an industrial perspective, these functional improvements should be balanced with considerations of scalability and cost, since solvent-based fractionation increases processing complexity. Future work should therefore explore long-term stability aspects (ice recrystallization and meltdown behavior) and assess the techno-economic feasibility of employing phospholipid fractions in large-scale ice cream production, as well as potential synergistic combinations of natural stabilizers and phospholipids to fully replicate the performance of synthetic emulsifiers.

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Declarations

Conflict of Interest The authors declare no conflict of interest.

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