Combination of milk polar lipids and casein hydrolysate as a healthy emulsifier for ice cream

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ABSTRACT

The demand for healthy ingredients in food products including ice cream, is continuously increasing. The potential of a combination of milk polar lipids (**MPL**) and casein hydrolysate (**CH**) to replace synthetic emulsifiers such as diacetyl tartaric acid esters of monoglycerides (DATEM), in ice cream production was investigated. Changes in particle size, emulsion stability, and interfacial tension of model emulsions (milk protein, casein:whey = 8:2, w/v) were analyzed after the addition of MPL, CH, and their combination (MPL+CH). The use of MPL+CH reduced interfacial tension and increased α_s- and β-casein displacement from the surface of cream layers compared to the addition of MPL alone. The addition of MPL+CH improved ice cream overrun to levels comparable to those of control ice cream containing DATEM (0.3%, w/v), without adversely affecting melt rate or microstructure. Confocal laser scanning microscopy revealed that ice cream prepared with MPL+CH formed a thick protein and coalesced fat layer on the surface of air cells that might help enhance overrun. These findings suggest that the combination of MPL (0.3%, w/v) and CH (0.03%, w/v) can be used as a potential emulsifier alternative to replace chemically synthesized emulsifiers such as DATEM.

Keywords**:** ice cream; casein hydrolysate; milk polar lipid, emulsifier; protein displacement

Introduction

Ice cream is a complex food colloid containing emulsion, foam, and dispersed ice crystals in a viscous aqueous solution. Ice cream generally contains about 0.1-0.3% emulsifiers, which contribute to the aeration of ice cream (Goff, 1997a). Monoglycerides, monoglyceride derivatives, and polysorbates have been commonly used as ice cream emulsifiers, and these emulsifiers are selectively adsorbed to the oil-water interface over milk proteins due to their greater surface activity (Euston and Goff, 2019). This preferential adsorption of emulsifiers leads to protein displacement at the interface and induces partial destabilization of fat droplets during aging (Pelan et al., 1997). The fat globule network formed by partial coalescence stabilizes air cells (Goff, 1997b) and improves overrun, melt resistance, and shape retention of ice cream (Barford et al., 1991; Daw and Hartel, 2015). Thus, ice cream mixes without suitable emulsifiers tend to have a wet and weak body due to insufficient partial coalescence (Goff, 1997a). In this regard, the adsorption behaviors of emulsifiers onto fat surfaces followed by protein displacement, provide valuable insights for the prediction of ice cream quality.

Recently, there has been a growing demand to replace synthetic food additives with more natural and healthy ones. Not all but some emulsifiers, such as carboxymethylcellulose and polysorbate 80, influence the interactions of mucus with gut microbiota and increase the risk of gut inflammation in mice and *ex vivo* human microbiota models (Chassaing et al., 2015; Naimi et al., 2021).

Milk polar lipids (**MPL**) are constituents of milk fat globule membrane (**MFGM**) and encompass glycosphingolipids, glycerophospholipids, sphingolipids, and gangliosides (Bourlieu and Michalski, 2015). Prophylatic MFGM administration improved mucus barrier function and attenuated acute colitis in a mice model of inflammatory bowel disease by decreasing inflammatory cytokines (Wu et al., 2022). The administration of polar lipid-enriched MFGM also improved obesity-mediated glucose metabolism disorders in rats (Li et al., 2022). These results suggest that MPL intake has beneficial

effects on the modulation of gut microbiota.

The interactions of MPL and dairy proteins are complex, and the effect on emulsion stability can vary depending on the type and concentration of proteins (Ahn et al., 2022). Milk phospholipid concentrate was able to competitively displace proteins from the surface of whey protein isolate (**WPI**)- stabilized emulsion (Livney et al., 2017). These studies suggest that interactions between MPL and milk proteins lead to partial coagulation of fat droplets by limiting protein layer formation at the emulsion interface. However, various single-ingredient emulsifier substitutes such as phospholipid-enriched whey protein concentrate, citrus fibers, rice protein contrate, and lupine protein concentrate did not achieve acceptable ice cream quality compared to mono- and diglycerides (**MDG**), which are commonly used as ice cream emulsifier (Loffredi et al., 2021). The authors suggest that a combination of different ingredients may be a promising strategy to replace MDG.

Protein hydrolysates generally display a better affinity for the oil/water interface than corresponding intact proteins because of increased hydrophobic amino acids exposure and molecular flexibility. We hypothesize that an appropriate combination of MPL and casein hydrolysate (**CH**) will improve partial coalescence and overrun in ice cream as well as provide a health benefit due to MPL. To prove this hypothesis, the effects of a combined MPL and CH (MPL+CH) formulation on protein displacement, emulsion stability, and interfacial tension were analyzed using a model milk protein-stabilized emulsion. Finally, the effects of this emulsifier combination on ice cream characteristics, such as microstructure, overrun, and melting, were evaluated.

Materials and Methods

Materials

Diacetyl tartaric acid esters of monoglycerides (**DATEM**) (Danisco, Denmark), MPL (DS-WPL

25; > 25% phospholipid; Solus biotech, Korea), and CH (90% protein,: 9% degree of hydrolysis, molecular weight distribution: 55.9% < 5,000 Da; 37.5% 5,000-20,000 Da; 6.6% > 20,000 Da; Tatua, New Zealand) were used as emulsifiers. Sodium caseinate (85% protein) and WPI (89% protein) were obtained from Lactoprot (Germany) and Lactalis Ingredients (France), respectively. Medium chain triacylglycerol (**MCT**) was purchased from Danisco. Ingredients used for ice cream preparation, including milk cream (Seoul Dairy Co-op., Korea), skim milk powder (Seoul Dairy Co-op.), corn syrup (Daesang Co., Korea), and sugar (Samyang Co. Ltd., Korea) were purchased from a local super market in Seoul, Korea.

Preparation of model emulsion

Milk protein (1%, w/v, sodium caseinate: WPI=8:2) or milk protein and CH (0.01%/0.05%/0.1%, w/v) were dispersed in sodium phosphate buffer (10 mM, pH 7.0) while MPL (1%, w/v) and DATEM (1%, w/v) were dispersed in MCT oil. After mixing the oil and protein phase, the mixture was blended using an Ultra-Turrax T25 homogenizer (IKA, Staufen, Germany) at 8,000 and 13,000 rpm for 1 min, respectively. The coarse emulsion was subjected to high-pressure homogenization using an NLM 100 Nano Disperser (Ilsin Autoclave, Korea) at 18/4 MPa. The emulsions were immediately cooled in an ice bucket and then stored at 4℃.

Preparation of ice cream

Ice cream mixes containing different emulsifiers were prepared, as shown in **Table 1**. After blending, the ice cream mix was pasteurized (72℃, 3 min) and passed through a 2-stage homogenizer (GEA Niro Soavi, Italy) at 18 MPa for the first stage and 3.4 MPa for the second stage. The homogenized mix was cooled in ice water and aged in a refrigerator at 4℃ for 18 h. Ice cream was

made using an ice cream machine (ISI-151TGN, Icetro, Korea). Ice cream was hardened in a deep freezer (-80°C) for 6 h and stored in a freezer at below -20°C.

Characterization of emulsion

Particle size analysis

The volume-weighted mean diameter (**D**[4,3]) of the emulsion was measured 1 h after emulsion formation using a Horiba LA-960 (Horiba Instruments, Japan). Refractive indices of oil (1.520) and deionized water (1.330) were used for calculation. The particle size distribution of aged ice cream was analyzed after appropriate dilution with distilled water. In the ice cream, the cumulative percentage of particles > 4.0 μm was considered as the index of partial fat coalescence because no globules > 4.0 μm were present in the mix (Bolliger et al., 2000). All determinations were carried out in triplicate.

Emulsion stability

The centrifugal stability constant was determined using the method of Liu et al (2022) with some modifications. After 1 h of aging at 4°C, emulsions (2 mL) were centrifuged at 3,000×g for 15 min. The supernatant (0.5 mL) was obtained after dilution (20-fold with distilled water) followed by centrifugation. The absorbance (A) was measured at 450 nm using a UV–visible spectrophotometer (Ultrospec 2100 pro, Amersham Biosciences, Uppsala, Sweden). The absorbance of the emulsion without centrifugation (A_0) was recorded, and the centrifugal stability constant (K_E) was calculated using the following formula:

$$K_E(\%) = \left|\frac{A_0 - A}{A_0}\right| \times 100$$

138 ***Interfacial tension***

139 The interfacial tension between the protein solution and oil was determined according to the
140 pendant drop method using KRÜSS drop-shape analyzer (DSA 25; GmbH, Germany). Emulsifiers
141 (milk protein, DATEM, MPL, CH, and MPL+CH) were solubilized in the aqueous phase, and MCT
142 oil was used as the oil phase. The concentrations of protein and emulsifiers were the same as emulsion
143 as described in the emulsion preparation. A syringe needle with a diameter of 1.825 mm was used to
144 create a pendant drop of MCT oil. Individual oil drop images were obtained using a high-speed digital
145 camera, and interfacial tension (mN/m) was calculated from the shape of the drop using the
146 Young−Laplace equation. Interfacial tension was determined at ambient temperature in triplicate.

147

148 **Protein displacement by emulsifiers**

149 The protein displacement of the model emulsion was determined using the method of Chen et al.
150 (2019a) with a slight modification. Samples (30 mL) were centrifuged (15,000xg, 45 min, 4℃) and
151 the cream layers were recovered after carefully separating the aqueous phase. In the case of ice cream,
152 cream layers were separated after thawing ice cream (4 mL) and an additional washing step was
153 conducted with distilled water. The adsorbed protein content in the cream layers (fat globule surface
154 protein) was calculated as the difference between the initial and aqueous protein content. The protein
155 content was quantified by the bicinchoninic acid assay (Smith et al., 1985). Protein displacement was
156 expressed using the following formula:

157 $$\text{Protein displacement } (\%) = \frac{M_0 - M_1}{M_0} \times 100$$

158 M_0: amount of fat globule surface protein in the absence of emulsifier (mg), M_1: amount of fat globule
159 surface protein in the presence of emulsifier (mg).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of cream layers

The separated cream layers were dispersed in sodium phosphate buffer (100 mM, pH 7.0) and heated at 95℃ for 20 min. The samples were mixed with the sample buffer and then boiled for 3 min and centrifuged (12,000 x g, 5 min) to remove residual fat. The samples (20 μL) were loaded onto a 12 % acrylamide gel (TGX Stain-Free™ FastCast™ Acrylamide Kit, Bio-Rad, CA, USA), and electrophoresis was conducted at a voltage of 80 V. The gel was visualized using a ChemiDoc™ XRS+System and relative intensity of α_s- and β-casein was quantified by Image Lab™ Software (ver. 5.1; Bio-Rad).

Quality characteristics of ice cream

Overrun

Ice cream mix and ice cream were filled in the same container (280 mL) and overrun was measured based on the weight difference between ice cream mix and frozen ice cream using the following formula:

$$\text{Overrun}\ (\%) = \frac{\text{Mix wt (g)} - \text{ice cream wt (g)}}{\text{Ice cream wt (g)}} \times 100$$

Melting rate

The melting rate of hardened ice cream was determined using the screen drip-through test (Muse and Hartel, 2004). The samples (72 g) were placed on a 20-mesh grid and allowed to stand at ambient temperature (25°C). The liquified samples that dripped through the grid were collected and their weight was recorded every 5 min for 70 min. The melting rate was calculated by plotting the drip weight as a function of time, and the slope of the linear part of the curve was expressed. The melting rate test was

182 determined in triplicate.

183

184 **Microstructure of ice cream**

185 The microstructure of thawed ice cream was observed using confocal laser scanning microscopy
186 (**CLSM**) (Leica, Heidelberg, Germany), as described by Lee et al. (2023). Fat and protein were stained
187 with Nile Red and Fast Green (0.1% and 0.01% in ethanol), respectively. For image analysis, samples
188 (200 μL) were immobilized by the addition of agarose solution (0.5%, 1:1, v/v) and visualized as
189 previously described (Ahn et al., 2022).

190

191 **Statistical analysis**

192 All analytical assays were carried out at least in triplicate. Data are presented as mean ± standard
193 deviation (SD). Statistical differences were analyzed by one-way analysis of variance (ANOVA) using
194 SPSS software (ver. 26 for Windows, SPSS Inc.; Armonk, NY, USA). If ANOVA indicated a significant
195 difference ($p < 0.05$), Tukey's multiple comparison test was used to compare significant differences
196 between treatment means.

197

198 ## Results and Discussions

199 **Changes in particle size and emulsion stability of model emulsion by the addition of emulsifiers**

200 The mean particle diameters of milk protein (casein:whey = 8:2)-stabilized emulsions (control
201 emulsion) with or without emulsifiers are shown in **Table 2**. The mean particle diameter of the control
202 emulsion was significantly decreased upon the addition of DATEM or MPL, respectively ($p<0.05$).

There was no significant difference in the mean particle diameter between the DATEM and MPL emulsions. The effect of CH addition on the mean particle diameter of emulsions varied depending on the additive level. CH at a low concentration (CH_L, 0.01%, w/v) did not affect the mean particle diameter, whereas CH at a high concentration (CH_H, 0.05%, w/v) significantly increased the mean particle diameter from 1.67 to 2.80 μm. The combination of MPL and CH decreased the mean particle diameter of emulsions compared to that of the CH counterpart, but the addition of MPL+CH_H increased the mean particle diameter compared to that of the control emulsion.

Centrifugal stability constants are calculated by the difference in absorbance before and after centrifugation of emulsions. Thus, a low stability constant denotes high emulsion stability. The emulsions containing CH_H with the larger particle size displayed a greater stability constant than the control emulsion. This result supports that the addition of high concentrations of CH caused significant destabilization of the emulsion.

The addition of DATEM (0.125~0.250%) significantly decreased the mean particle diameter of whey protein-maltodextrin-stabilized emulsion and improved emulsion stability (Yu et al., 2021). Low molecular weight emulsifiers (**LMWE**) are able to locate at the interface together with protein, such as caseinate, and fill up the holes of the interfacial protein film until LMWE-mediated protein displacement take place (Munk et al., 2014).

Generally, limited enzymatic hydrolysis is applied in commercial protein hydrolysate production to minimize the risk of bitter taste development. Protein hydrolysis enhances surface adsorption kinetics by increasing exposure of hydrophobic groups (Chen et al. 2019b). Resistance of fat droplets to coalescence tends to be positively correlated with the amount of peptide > 2 kDa (van der Ven et al., 2001). Similarly, protein hydrolysates with a relatively shorter chain length (< 5 kDa) form a weak interfacial film, resulting in low emulsion stability (Schroder et al., 2017). This is partially explained

by the fact that extensive hydrolysis increases the aqueous solubility of peptides rather than their adsorption at the oil-water interface (Conde and Patino, 2007).

Changes in interfacial tension by the addition of emulsifiers

The changes in interfacial tension following the addition of DATEM, MPL, and MPL+CH were measured. Aqueous protein (control) decreased the interfacial tension by about 12 mN/m and the addition of DATEM (1%, w/v) and MPL (1%, w/v) significantly lowered the interfacial tension, suggesting DATEM and MPL can be preferentially adsorbed at the oil-water interface ($p<0.05$, **Fig. 1**). LMWE might fill up gaps in the interfacial protein network that are not covered by larger proteins (Makie et al., 2000). DATEM actively reduced the interfacial tension of aqueous milk protein and displayed the greatest interfacial tension-lowering effect. The addition of CH also significantly reduced the interfacial tension even at a low additive level (0.01%. w/v). When MPL+CH was applied the interfacial tension was further decreased compared to MPL alone. Emulsifiers with high interfacial tension-lowering effect can be more densely packed at emulsion surface (Bezelgues et al., 2008). In this regard, MPL+CH facilitates the formation of a mixed interfacial MPL+CH layer, and the combined presence of MPL and CH leads to changes in interfacial viscoelasticity, affecting emulsion destabilization. Dalgleish et al. (1995) studied the surface interaction of casein and Tween 80 in an oil-in water emulsion. They found that the protein-emulsifier interactions not only affected the amount of adsorbed protein but also its conformation at the interface until equilibrium between aqueous protein and adsorbed protein was established.

Effect of emulsifier addition on protein displacement from emulsion cream layers

The addition of LMWE often leads to displacement of interfacial adsorbed proteins, followed by

249 partial coalescence of fat globules (Goff, 1997b). The effect of emulsifier addition on milk protein
250 displacement from cream layers was indirectly determined by quantification of aqueous proteins after
251 cream separation, and profiles of protein remaining in the cream layers was examined using SDS-
252 PAGE.

253 The intensity of casein bands remaining in the emulsion cream layers decreased as the
254 concentration of CH increased. Caseins in the cream layers was almost completely replaced by low-
255 molecular-weight casein peptides when 0.05% or 0.1% CH was added. In addition, CH (0.01%) more
256 effectively displaced β-casein than α_s-casein (**Fig. 2**). β-casein is the most abundant and surface-active
257 casein and adsorbed β-casein at the interface stabilizes an emulsion by providing thickness and steric
258 hindrance (Li et al., 2016). The low emulsion stability observed with CH_H addition (**Table 2**) might
259 be associated with the desorption of β-casein. Consequently, the combination of MPL+ CH (> 0.05%)
260 was excluded from further ice cream application experiments because the emulsion in ice cream should
261 have good stability in the stationary state before freezing and it provide sufficient instability under
262 shear conditions during freezing in ice cream production (Goff, 1997b).

263 From the SDS-PAGE protein profile, the intensity of major casein constituents, α_s and β-casein,
264 was decreased by the addition of other emulsifiers (**Fig. 3**). DATEM more greatly expelled α_s- and β-
265 casein from the cream layer surface when compared with MPL. The intensity of SDS-PAGE bands
266 corresponding to casein and CH was further decreased when the MPL+CH combination was used. The
267 presence of both LMWE and proteins influences emulsion stability depending on the concentration
268 and type of emulsifiers/proteins as well as the sequence of addition. LMWE diffuse without restriction
269 above the melting temperature. Thus, an emulsion interface occupied by LMWE exert less surface
270 mechanical properties than that of protein-rich interface (Wilde et al., 2004). DATEM has a better
271 affinity for the oil surface and is more likely to displace caseins adsorbed at the oil-water interface.

DATEM (melting point: ~45°C) crystalizes from melt to a stable α-crystal form at the interface at high surfactant concentration, and these crystals promote penetration of the interfacial film to enhance partial coalescence (Hong, 1998). In addition, repulsive forces between the net negative (-) charge of casein emulsion and that of carboxyl groups in DATEM at pH 7.0 facilitate casein dispersion from the interfacial surface. Electrostatic interaction between adsorbed milk proteins and phosphatidylcholine, one of the major constituents of MPL has been demonstrated (Allen et al., 2008).

As shown in **Fig. 4**, the addition of DATEM (1%) more effectively displaced milk proteins from the cream layers when compared with MPL (62% vs. 29%). The combination of MPL+CH_L (0.01%) increased protein displacement (29% vs. 46%). Therefore, the combination of MPL+CH desorbed milk proteins at the interface in a synergistic manner without critically affecting stability in the static state.

Effect of MPL and CH combination on quality attributes of ice cream

During the homogenization of an ice cream mix, surface-active ingredients, such as proteins and emulsifiers, will adsorb at the oil-water interface. The formation of composite emulsion layers and the partial coalescence of fat play an important role in the quality attributes of ice cream. The concentration of MPL (0.15% vs. 0.3%) was selected to remain below the emulsifier concentration (about 0.5%) generally used in ice cream production, considering cost and sensory characteristics.

The addition of combined MPL+CH (L/M/H) significantly improved ice cream overrun (41~43%) when compared with MPL alone (35%). The overrun of ice cream prepared with MPL (0.3%)+CH was comparable to that produced with DATEM (44%) as the emulsifier (**Table 3**). Higher overrun indicates that air is well distributed and retained in the ice cream structure. The increased overrun observed with the combination of MPL+CH is likely due to enhanced protein displacement from the emulsion surface,

294 as shown in Fig. 4. CH, which has a relatively low MW and a flexible structure, may competitively
295 adsorb at the oil-water interface, thereby reducing the surface coverage casein micelle. The decreased
296 steric stabilization, resulting from limited casein micelle adsorption, leads to the formation of partially
297 coalesced fat networks that confer excellent air cell stabilization property (Goff, 2016). In ice cream
298 structure formation, LMWE gradually displaces milk proteins from the fat globule surfaces during
299 freezing and develops fat globule clusters to stabilize foam structure (Warren and Hartel, 2018). Chen
300 et al. (2019a) reported that protein displacement from the fat surface decreases the mechanical strength
301 of the adsorbed layers and leads to partial coalescence fat globules. Under shear conditions, protein
302 displacement is dependent on the type and concentration of emulsifiers (Davies et al., 2001).

303 LMWE not only displace proteins from the fat surface but also play an important role in fat
304 crystallization. Water insoluble emulsifiers such as mono- and diglycerides, act as templates for fat
305 crystallization, whereas water soluble Tween 80 forms loosely packed weak crystals (Rizzo et al.,
306 2015). Fat crystals are able to penetrate interfacial layers and bridge fat droplets to form fat globule
307 clusters and improve overrun. In this regard, MPL promotes fat crystal penetration with relatively weak
308 protein displacement activity, while CH further facilitates protein desorption from the ice cream
309 emulsion interface.

310 Differences in the melting rate of ice cream samples hardened for 6 h were compared. The melting
311 rate of ice cream prepared with different emulsifier formulations did not show significant difference
312 compared to control ice cream containing DATEM. It is known that ice cream with low overrun tends
313 to melt quickly because air cells in ice cream act as a barrier against heat transfer (Muse and Hartel,
314 2004). The three-dimensional fat globules network stabilizes air cells by attachment to the bubble
315 surfaces in ice cream and prevents serum drainage during melting, whereas ice cream with a less
316 developed fat network exhibits rapid serum dripping (Koxholt et al., 2001; Wu et al., 2019).

317 The percentage of fat globules > 4 μm was used as an index of the extent of partial coalescence.
318 The ice cream prepared with MPL (0.3%)+CH_H (0.03%) showed significantly more fat aggregates >
319 4 μm (%) than the other ice creams, which did not show significant differences from the control ice
320 cream. (**Table 3).** Mendez-Velasco and Goff (2012) reported that fat-monoglyceride interaction in ice
321 cream were altered by the degree of saturation of the monoglyceride. Changes in fat-emulsifier
322 interactions influence ice cream quality by modifying the size and quantity of fat aggregates.

323 The effect of MPL+CH on protein displacement after ice cream production was determined.
324 DATEM displayed the highest protein displacement (52%), and MPL (0.3%) alone showed the lowest
325 protein displacement (13%) (**Table 3**). Protein displacement increased by the use of MPL+CH
326 combination. However, CH concentration in the combination had no significant effect on the protein
327 displacement.

328 Emulsifiers used in ice cream promote protein displacement, especially casein micelles, from the
329 fat-water interface and improve sensitivity to partial coalescence upon shearing (Goff, 2016). The air-
330 water interface is not completely covered by partially-coalesced fat globules, and proteins adsorbed to
331 the air-interface also play an important role in the aeration of ice cream (Zhang and Goff, 2004).
332 According to a study of immunogold-labeled β-casein adsorption to the air interface in ice cream, the
333 dissociation of casein micelles to soluble casein by EDTA improved protein adsorption at the air
334 interface (Zhang and Goff, 2004). Therefore, in the context of ice cream production, CH might have a
335 greater affinity for the air interface than casein micelles. Taken together, ice cream added with
336 MPL+CH improved both protein displacement and partial coalescence when compared to MPL alone.
337
338

Microstructure of air cell interface in ice cream containing MPL and CH

The structure of fat globules surrounding air cells in thawed ice cream was observed using CLSM. The representative image of control ice cream containing DATEM was compared with ice cream produced using a combination of MPL (0.3%)+CH (0.03%) because it showed comparable ice cream quality to the control ice cream. In the CLSM images, fat globules are stained red and the proteins are stained green (**Fig. 5**). There was no distinct difference among the ice cream mix samples, but there was some variation in the size of fat particles located on the air cell interfaces among the ice cream samples. Partially coalesced fat globules uniformly surrounded air cells in the control ice cream, whereas ice cream containing MPL+CH formed relatively heterogeneous larger-sized fat globules at the air cell interface. Although air cell surface coverage was not as uniform as the control ice cream, MPL+CH developed a thick layer of protein and coalesced fat on the air cell surface that might help enhance overrun. These results suggests that MPL+CH can be used as a potential emulsifier alternative to replace chemically synthesized emulsifiers such as DATEM.

Conclusion

The displacement of milk proteins from the emulsion droplet surface was significantly improved by the combination of MPL+CH compared to MPL alone. The increased milk protein displacement promoted partial coalescence of fat globules and increased ice cream overrun. The overall quality attributes of ice cream prepared using a combination of MPL+CH were comparable to those of the control ice cream. Therefore MPL+CH can be used as a healthy emulsifier in ice cream production.

Conflicts of Interest

Industry employees are involved in ice cream preparation but they had no role in the interpretation of data or publication processes.

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Author Contributions

Conceptualization: Imm J-Y, Lee S h, Data curation: Park J-H, Lee Y-B, Investigation: Park J-H, Ko, E, Writing - original draft: Park J-H, Writing - review & editing: Imm J-Y, Lee S h, Park J-H, Lee Y-B, and Ko E.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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469 **Table 1. Formulation of ice cream mix**

470

Ingredients (%)			MPL (0.15%)			MPL (0.3%)		
	DATEM	MPL	CH_L	CH_M	CH_H	CH_L	CH_M	CH_H
Fat	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
MSNF	10.80	10.80	10.80	10.80	10.80	10.80	10.80	10.80
Sugar	9.50	9.50	9.64	9.63	9.62	9.50	9.50	9.50
Syrup	6.50	6.50	6.50	6.50	6.50	6.50	6.50	6.50
DATEM	0.30	-	-	-	-	-	-	-
MPL	-	0.30	0.15	0.15	0.15	0.29	0.28	0.27
CH	-	-	0.01	0.02	0.03	0.01	0.02	0.03
Stabilizer	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Water	60.6	60.6	60.6	60.6	60.6	60.6	60.6	60.6
Total	100	100	100	100	100	100	100	100

471

472 MSNF, Milk solid not fat; DATEM, diacetyl tartaric acid esters of monoglycerides (0.3%, w/v); MPL,
473 milk polar lipids (0.3%, w/v); CH_L/M/H, casein hydrolysate (0.01%/0.02%/0.03%, w/v)

474

475 **Table 2. Changes in mean particle diameter and centrifugal stability contents of milk protein-**
476 **stabilized emulsion by addition of emulsifiers**
477

						MPL	
	CON	DATEM	MPL	CH_L	CH_H	CH_L	CH_H
Mean particle diameter (μm)	1.67±0.01^{c}	1.33±0.02^{de}	1.26±0.01^{e}	1.60±0.06^{cd}	2.80±0.30^{a}	1.55±0.01^{cde}	2.09±0.11^{b}
Centrifugal Stability constants (%)	65.4±1.5^{ab}	58.3±0.9^{bc}	59.2±3.3^{bc}	63.0±4.8^{ab}	69.6±2.9^{a}	51.3±8.1^{c}	69.9±2.9^{a}

478
479 Con, milk protein (casein:whey = 8:2, 1%, w/v); DATEM, diacetyl tartaric acid esters of
480 monoglycerides (1%, w/v); MPL, milk polar lipids (1%, w/v); CH_L/H, casein hydrolysate
481 (0.01%/0.05%, w/v). Different superscript (a–e) in the same row indicate significant differences at
482 $p<0.05$. Error bars indicate SD of triplicate measurements.
483

Table 3. Changes in overrun, melting rate, volume of fat droplets > 4 μm, and protein displacement in ice cream by emulsifiers.

			MPL (0.15%)			MPL (0.3%)		
	DATEM	MPL (0.3%)	CH_L	CH_M	CH_H	CH_L	CH_M	CH_H
Overrun (%)	44.0±3.2^{a}	34.8±1.9^{b}	37.8±3.1^{ab}	39.7±1.3^{ab}	40.2±3.6^{ab}	41.2±5.0^{a}	40.6±2.4^{ab}	43.1±2.7^{a}
Melt rate	2.22±0.06^{a}	2.22±0.02^{a}	2.20±0.01^{a}	2.20±0.02^{a}	2.26±0.04^{a}	2.22±0.03^{a}	2.22±0.10^{a}	2.18±0.06^{a}
Fat globules > 4 μm (%)	5.27±0.94^{b}	8.53±1.23^{b}	9.45±2.58^{b}	11.78±1.05^{b}	9.27±0.70^{b}	9.83±1.74^{b}	9.2±4.75^{b}	20.01±0.96^{a}
Protein displacement (%)	51.7±2.04^{a}	12.99±0.6^{d}	19.31±2.71^{bcd}	20.56±2.57^{bcd}	26.65±4.04^{bc}	18.3±3.31^{cd}	20.34±8.38^{bcd}	30.35±6.03^{b}

DATEM, diacetyl tartaric acid esters of monoglycerides (0.3%, w/v); CH_L/M/H, casein hydrolysate_0.01%/0.02%/0.03% (w/v); Different superscript (a–e) in the same row indicate significant differences at $p < 0.05$. Error bars indicate SD of triplicate measurements.

Figure Captions

Fig. 1. Changes in interfacial tension of milk protein solution by addition of emulsifiers. Con**,** milk protein (1%, w/v: casein:whey = 8:2); DATEM, diacetyl tartaric acid esters of monoglycerides (1%, w/v); MPL: milk polar lipids (1%, w/v); CH, casein hydrolysate (0.01%, w/v). Different letters (a–e) indicate significant differences at $p < 0.05$. Error bars indicate SD of triplicate measurements.

Fig. 2. Effect of CH addition on protein profile of cream layers of milk protein-stabilized emulsion. Con**,** milk protein (1%, w/v: casein:whey = 8:2); CH, casein hydrolysate (0.01%, 0.05%, 0.1%, w/v). Cream layers were separated by centrifugation of emulsion (15000 x g, 45 min at 4℃) and the profile of protein remaining in the cream layer was analyzed by SDS-PAGE.

Fig.3. Effect of various emulsifiers addition on protein profile of cream layers of milk protein-stabilized emulsion.

Con**,** milk protein (1%, w/v, casein:whey = 8:2); DATEM, diacetyl tartaric acid esters of monoglycerides (1%, w/v); MPL: milk polar lipids (1%, w/v); CH, casein hydrolysate (0.01%, w/v); MPL+CH, MPL (1%, w/v) + CH (0.01%). Cream layers were separated by centrifugation of emulsion (15000 x g, 45 min, 4℃) and the profile of protein remaining in the cream layer was analyzed by SDS-PAGE.

Fig. 4. Protein displacement of milk protein-stabilized emulsion by addition of emulsifiers.

Con**,** milk protein (1%, w/v: casein:whey = 8:2); DATEM, diacetyl tartaric acid esters of monoglycerides 1% (w/v); MPL, milk polar lipids 1% (w/v); CH, casein hydrolysate (0.01%, w/v).

516 Different letters (a–e) indicate significant differences at $p < 0.05$. Error bars indicate SD of triplicate
517 measurements.

518

519 **Fig. 5. Microstructure of fresh mix and air cell interface in ice cream containing MPL and CH**
520 **combination**

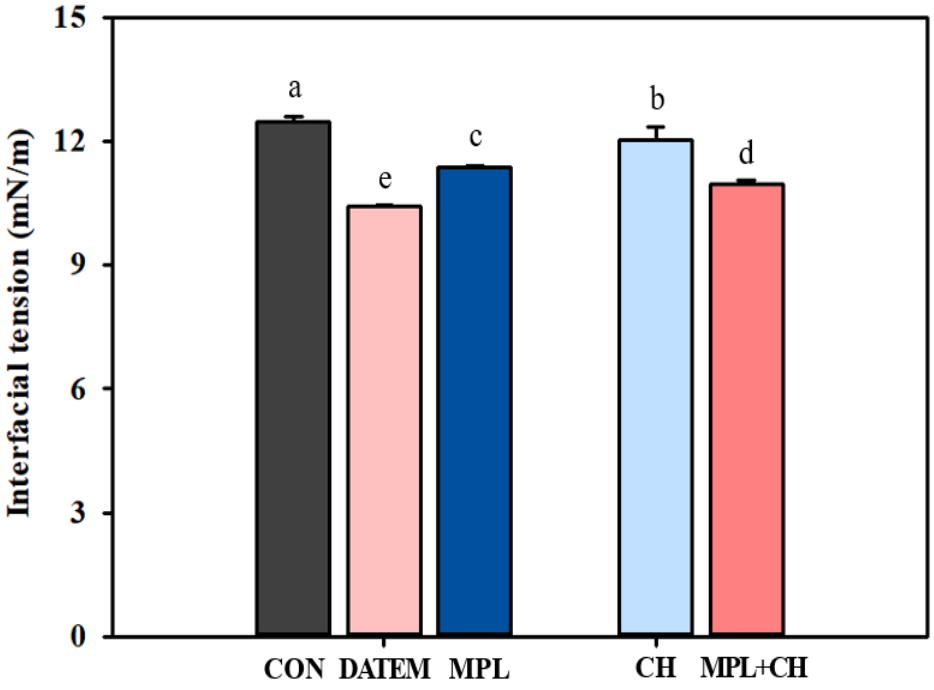
521 Fresh mix and thawed ice cream were observed using confocal laser scanning microscopy. DATEM,
522 diacetyl tartaric acid esters of monoglycerides (0.3%, w/v); MPL, milk polar lipids; CH, casein
523 hydrolysate; MPL+CH, MPL (0.3%, w/v) + CH (0.01%, w/v). Magnification: 126 x.

524

525

526 **Fig. 1**

527



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532 **Fig. 2**

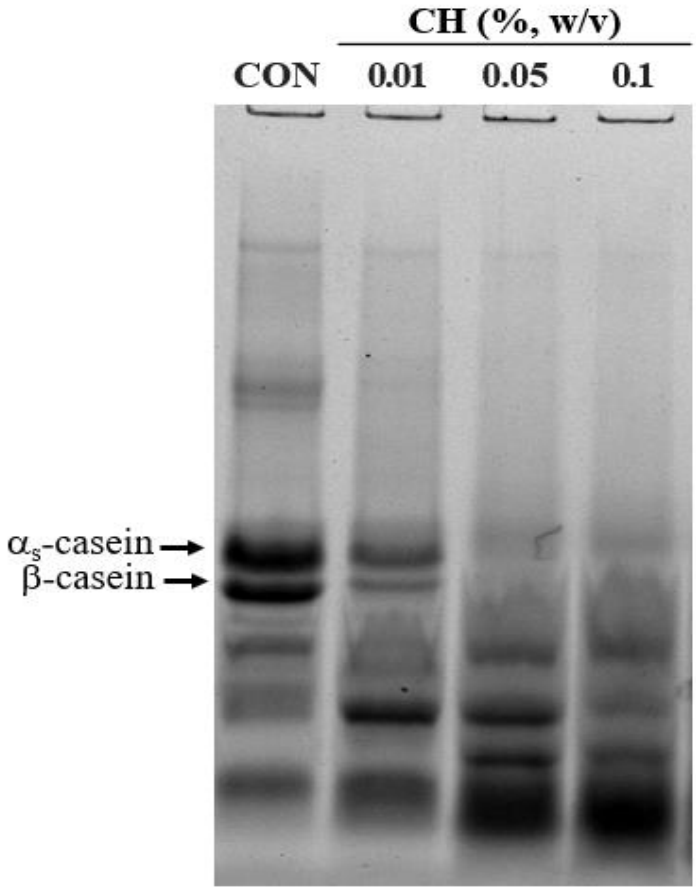

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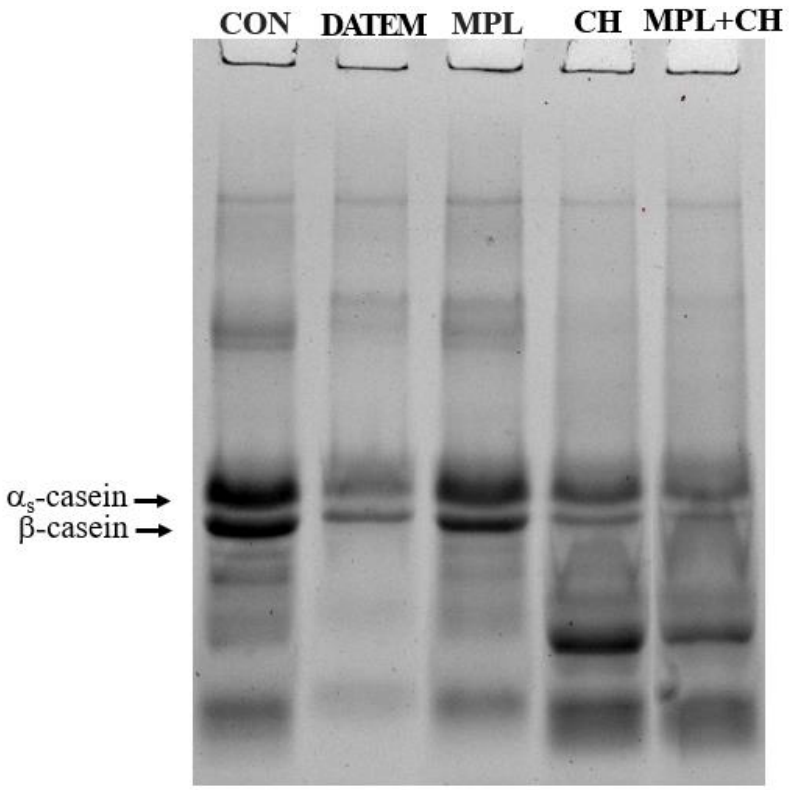
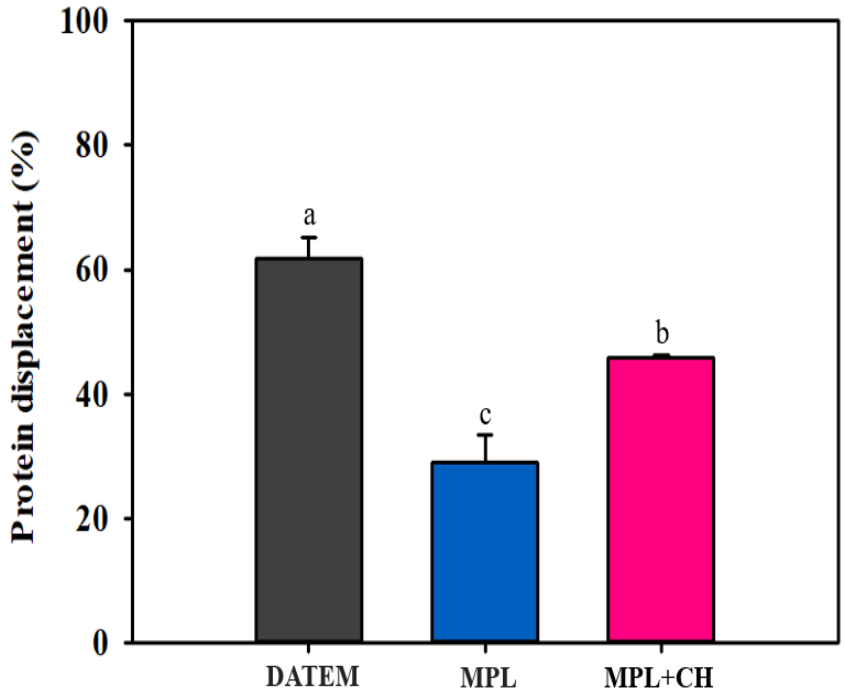
Fig. 3



543 **Fig. 4**



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547 **Fig. 5**

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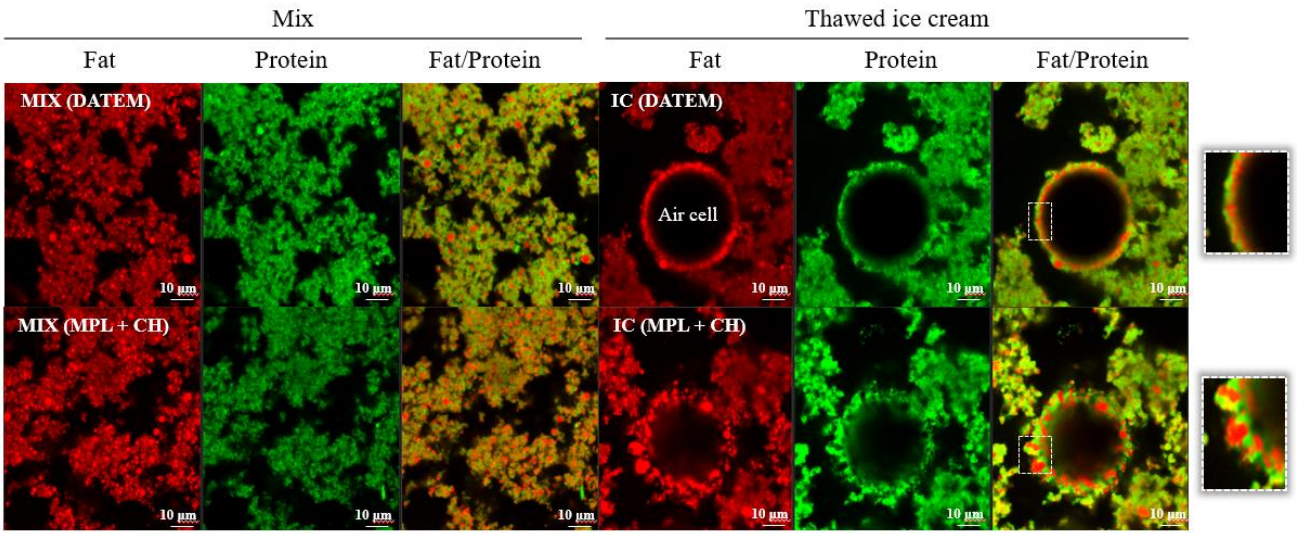

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