

# LWT

## Effects of different emulsifier substitutes on artisanal ice cream quality

--Manuscript Draft--

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<b>Abstract:</b>	<p>This work aims at assessing the performances of eight commercial emulsifier substitutes (not identified as additives) in an artisanal ice cream formulation, in comparison with the common mono- and di-glycerides of fatty acids (MDG). Besides the quality features of mixes (density, soluble solids, rheological properties, freezing point depression) and ice creams (extrusion parameters, overrun, firmness, melting behavior, shape retention), also the emulsifying activity index (EAI) and creaming stability (CS) of the considered ingredients were tested. No significant correlations were found between EAI or CS and quality characteristics of ice creams, confirming the singular role of MDG in ice cream structuring. Citrus fibers excessively increased the mix viscosity compared to the reference sample containing MDG (<math>435\pm 3</math> vs <math>89\pm 1</math> mPa s). Lupine, pea, and rice proteins, as well as citrus fibers significantly improved ice cream melting behavior (<math>1.60\pm 0.12</math> - <math>2.33\pm 0.01</math> g/min melting rate vs. <math>2.62\pm 0.05</math> g/min of the reference sample). However, at the tested doses there was not a single ingredient as effective as MDG on ice cream properties. A combination of different substitutes in different amounts can be a valuable strategy in order to effectively replace MDG and attain the goal of an ice cream with good quality and clean label.</p>



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Dear Editor,

Please find the second revision of the manuscript "Effects of different emulsifier substitutes on artisanal ice cream quality" by Loffredi E., Moriano M.E., Masseroni L, and Alamprese C. The only Reviewer's suggestion has been followed, dividing Results and Discussion in subsections based on the type of emulsifier substitute. Text changes are highlighted in red.

We do hope that you can appreciate our revision work, thus reconsidering your decision.

The work described has not been published previously, is not under consideration for publication elsewhere, and its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out; if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Looking forward to hearing from you soon, I remain sincerely yours.

Cristina Alamprese

**Ms. Ref. No.: LWT-D-20-04443R1**

Effects of different emulsifier substitutes on artisanal ice cream quality

Eleonora Loffredi, Maria Eletta Moriano, Letizia Masseroni, Cristina Alamprese

### **ANSWERS TO REVIEWER#1**

*Although the majority of my minor recommendations were addressed, the organization in the results and discussion was not improved. Again, I think the current organization is insufficient for publication in its current form as it very difficult to follow and hard to understand the treatments that are being compared. As suggested previously, I would recommend discussing each emulsifier separately and comparing them all to the control, but this is just a suggestion. The organization does need to be altered in a significant way though.*

The Reviewer's suggestion has been followed, completely re-writing the Results and Discussion section and organizing it in separated paragraphs for each emulsifier.

## **Highlights**

- Evaluation of eight commercial emulsifier substitutes for artisanal ice cream
- No correlations between substitutes' emulsifying properties and ice cream quality
- Citrus fibers excessively increased the mix viscosity
- Lupine, pea, rice proteins and citrus fibers improved ice cream melting behavior
- No single ingredient as effective as mono- diglycerides on ice cream properties

1                   **Effects of different emulsifier substitutes on artisanal ice cream quality**

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10

11 **Abstract**

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13 identified as additives) in an artisanal ice cream formulation, in comparison with the common  
14 mono- and di-glycerides of fatty acids (MDG). Besides the quality features of mixes (density,  
15 soluble solids, rheological properties, freezing point depression) and ice creams (extrusion  
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17 activity index (EAI) and creaming stability (CS) of the considered ingredients were tested. No  
18 significant correlations were found between EAI or CS and quality characteristics of ice  
19 creams, confirming the singular role of MDG in ice cream structuring. Citrus fibers  
20 excessively increased the mix **apparent** viscosity compared to the reference sample containing  
21 MDG ( $435\pm 3$  vs  $89\pm 1$  mPa s). Lupine, pea, and rice proteins, as well as citrus fibers  
22 significantly improved ice cream melting behavior ( $1.60\pm 0.12$  -  $2.33\pm 0.01$  g/min melting rate  
23 vs.  $2.62\pm 0.05$  g/min of the reference sample). However, at the tested doses there was not a  
24 single ingredient as effective as MDG on ice cream properties. A combination of different  
25 substitutes in different amounts can be a valuable strategy in order to effectively replace  
26 MDG and attain the goal of an ice cream with good quality and clean label.

27

28 **Keywords:**  $\alpha$ -cyclodextrin; citrus fibers; phospholipids; legume proteins; rice proteins.

29

30

31 **1. Introduction**

32 Ice cream is a frozen dairy product made of a complex matrix of fat clusters, milk solids non-  
33 fat, air bubbles, water, sweeteners, and, often, stabilizers, emulsifiers and flavors. Emulsifiers  
34 are usually added at a level of 0.1-0.3 g/100g and they are of primary importance in  
35 enhancing fat destabilization during freezing, favoring partial coalescence between the closest  
36 fat globules, so that air bubbles are stabilized with a homogeneous distribution resulting in the  
37 desirable ice cream structure and properties. Their fat-destabilization promoting mechanism is  
38 linked to the lowering of the fat/water interfacial tension in the mix, which promotes protein  
39 displacement from the fat globule surface, thus reducing the stability of the fat globule and  
40 allowing partial coalescence during whipping and freezing. The extent of protein  
41 displacement from the membrane is a function of the emulsifier type and concentration (Goff  
42 & Hartel, 2013).

43 The most widely used emulsifiers in ice cream are mono- and di-glycerides of fatty acids  
44 (MDG), which are indicated by the code E471 according to the European law about food  
45 additives (Reg. EC No 1333/2008). However, nowadays consumers are increasingly  
46 demanding “clean label” or “E-free” products, in which no additives indicated with the  
47 European E-number are used. Thus, also in the ice cream field, different substitutes have been  
48 recently tested for their abilities in conferring to the final product the well-appreciated quality  
49 and sensory properties (Levin, Burrington, & Hartel, 2016b; Ralla et al., 2018), and many  
50 ingredients are already available on the market.

51 A growing interest has been shown for legume proteins, which, as other proteins, can deliver  
52 interesting technological functionalities, such as emulsifying properties, because of their  
53 ability to be adsorbed at the surface of oil droplets, thus reducing the interfacial tension and  
54 preventing coalescence (Karaca, Low, & Nickerson, 2011). Several studies have  
55 demonstrated the emulsifying properties of proteins from lupine (Jayasena, Chih, & Nasar-

56 Abbas, 2010), pea (Tamm, Herbst, Brodkorb, & Drusch, 2016; Karaca et al., 2011), chickpea,  
57 fava beans, lentil, and soy (Karaca et al., 2011), but their use in ice cream needs to be better  
58 evaluated. Herald, Aramouni, and Abu-Ghoush (2008) used a soy protein isolate as  
59 emulsifying agent alternative to egg yolk in French vanilla ice cream, but the egg-control  
60 sample resulted in significantly higher softness and sensory acceptability.

61 Another category of possible substitutes for ice cream emulsifiers are phospholipids, which  
62 can be derived from different sources, such as milk, soy, or rice. Rinaldi et al. (2014) used  
63 them in ice cream production and, although the results were not wholly comparable to those  
64 of the reference formulation containing MDG in terms of overrun, unfreezable water, and fat  
65 destabilization, the authors encouraged further studies about the use of different phospholipids  
66 alone or in combination at different levels. Some authors (Bund & Hartel, 2013; Daw &  
67 Hartel, 2015; Levin et al., 2016b) suggested the use of whey protein concentrate enriched in  
68 phospholipids (named pro-cream) and delactosed whey permeate as ice cream ingredients in  
69 order to valorize these cheese-making by-products. Actually, emulsifier substitutes can be  
70 obtained from correctly treated food by-products or wastes, thus increasing the sustainability  
71 of the production chain. In this context, orange fibers recovered from the juice industry were  
72 used as fat replacers in ice cream (Crizel, Jablonski, Rios, Rech, & Flôres, 2013; Crizel,  
73 Araujo, Rios, Rech, & Flôres, 2014), whereas their emulsifying properties were scarcely  
74 investigated (Wallecan, McCrae, Debon, Dong, & Mazoyer, 2015).

75 Other interesting dietary fibers that can be used as emulsifier alternatives are cyclodextrins,  
76 which have a three-dimensional structure with the interior and exterior portions possessing  
77 hydrophobic and hydrophilic characteristics. A demonstration of their surface activity can be  
78 found in the studies by Davarpanah and Vahabzadeh (2012) and Eslami, Davarpanah, and  
79 Vahabzadeh (2017), where the emulsifying functionality of  $\beta$ -cyclodextrin was evaluated in  
80 O/W Pickering emulsions. However,  $\alpha$ -cyclodextrin emulsifying properties have not been

81 deeply studied so far, unless in pharmaceutical preparations (Leclercq & Nardello-Rataj,  
82 2016).  
83 Despite the high number of emulsifier substitutes present on the market, a comprehensive  
84 comparison of different commercial products for artisanal ice cream has not been published so  
85 far. Thus, the aim of this work was to assess the performances of eight possible commercial  
86 E-free emulsifier substitutes (i.e., whey proteins, whey phospholipids, soy, pea, lupine, and  
87 rice proteins, citrus fibers, and  $\alpha$ -cyclodextrin) in an artisanal ice cream formulation, in  
88 comparison to the common MDG. In addition to the mix and ice cream quality features,  
89 emulsifying properties (i.e., emulsifying activity index and creaming stability) of the  
90 considered ingredients were tested. The final aim is to provide ice cream producers with  
91 objective indications about the effects of all these ingredients on ice cream quality, in order to  
92 be able to find their way through the ingredient market.

93

## 94 **2. Materials and methods**

### 95 *2.1. Ice cream ingredients*

96 For ice cream production the following liquid and powdered ingredients were used:  
97 pasteurized whole milk (Granarolo Alta Qualità, Granarolo S.p.A., Milan, Italy), pasteurized  
98 cream (Centrale del Latte di Milano, Milan, Italy), skim milk powder, sucrose, dextrose,  
99 stabilizers (a mix of carboxymethylcellulose and tare gum usually used for artisanal ice cream  
100 production), and MDG. Powdered ingredients were kindly provided by Comprital S.p.A.  
101 (Settala, Italy).

102 The following commercial ingredients were used as emulsifier substitutes: whey protein  
103 concentrate (WPC 75, Lactoland, Dülmen, Germany; protein content, 75 g/100 g; code,  
104 WPC); whey protein concentrate with high phospholipid content (Lacprodan MFGM-10,  
105 Arla, Viby, Denmark; protein content, >69 g/100 g; phospholipid content, 6-8 g/100 g; code,

106 WPP); soy protein isolate (Ipsos-CHN, Vaessen-Schoemaker B.V., Deventer, Netherlands;  
107 protein content, 90 g/100 g; code, SPI); pea protein concentrate (Pea Protein 80%  
108 Conventional, Barentz Service S.p.A., Paderno Dugnano, Italy; protein content, 81.3 g/100 g;  
109 code PPC); lupine protein concentrate (FRALU-CON, Frank Food Products, Twello,  
110 Netherlands; protein content, 60 g/100 g; code, LPC); rice protein concentrate (Remypro  
111 N80+, Beneo-Remy N.V., Leuven-Wijgmaal, Belgium; protein content, 79 g/100 g; code  
112 RPC); citrus fibers (Citri-Fi 100M40; FiberStar, River Falls, WI; code CF);  $\alpha$ -cyclodextrin  
113 (Cavamax W6 Food, Wacker Chemie AG, Munich, Germany; code ACD).

114

## 115 *2.2. Characterization of emulsifier substitutes*

### 116 *2.2.1. Protein content*

117 For protein-based emulsifier substitutes, the protein content was determined according to the  
118 AOAC Official Method 920.87 (AOAC, 1999), adopting a nitrogen/protein conversion factor  
119 of 6.25 for SPI, PPC, RPC, and LPC, and of 6.38 for WPC and WPP. Results (g/100 g) are  
120 expressed as the average and standard deviation values of four replicates.

121

### 122 *2.2.2. Emulsifying activity index*

123 Emulsifying activity index (EAI) was determined according to the method described by Mu,  
124 Zhao, Zhao, Cui, and Liu (2011) with slight modifications. Briefly, for MDG and all the  
125 emulsifier substitutes, a 0.5 g/100 mL solution in 50 mmol/L sodium phosphate buffer (pH 7)  
126 was prepared. After stirring for 1 h at 25 °C, 6 mL solution was emulsified with 2 mL corn oil  
127 (Carrefour, Boulogne-Billancourt, France) at 7200 rpm for 5 min using a T25 digital Ultra  
128 Turrax (IKA, Staufen, Germany). The obtained emulsion (200  $\mu$ L) was diluted 1:25 in 10  
129 mmol/L sodium phosphate buffer (pH 7) containing 0.1 g/100 mL sodium dodecyl sulphate  
130 (Sigma Aldrich, Saint Louis, MO). The absorbance was then measured at 500 nm using a V-

131 650 spectrophotometer (Jasco Europe, Cremella, Italy). EAI (m<sup>2</sup>/g) was calculated by  
132 following Equation 1:

$$133 \quad EAI = \frac{2 \cdot 2.303 \cdot A \cdot N}{c \cdot \varphi \cdot l \cdot 10000} \quad (\text{Eq. 1})$$

134 where  $A$  is the absorbance of the diluted emulsion,  $N$  is the dilution factor (25),  $c$  is the  
135 concentration of the tested ingredient in the emulsion (g/mL),  $\varphi$  is the oil volume fraction of  
136 the emulsion (0.25), and  $l$  is the path length (0.01 m). Results are expressed as the average and  
137 standard deviation values of four replicates.

138

### 139 2.2.3. Creaming stability

140 Creaming stability (CS) was determined as described by Moriano and Alamprese (2020).

141 Briefly, Oil Red O (Sigma Aldrich, Saint Louis, MO) was added (0.0015 g/100 g) to corn oil  
142 (Carrefour, Boulogne-Billancourt, France). Oil-in-water emulsions (20 mL) were prepared as  
143 previously described for EAI measurement, but with a 4:1 ratio water phase:dyed oil.

144 Immediately after preparation, the emulsions were transferred into 10 mL graduated glass  
145 cylinders and stored for 1 h at 25 °C. The emulsion stability was assessed by measuring the  
146 bottom turbid layer separated from the red cream layer. Results are expressed in percentage,  
147 as the average and standard deviation values of two measurements.

148

### 149 2.3. Ice cream formulations

150 Ice cream formulations were developed based on a standard artisanal recipe (Table 1). The  
151 reference ice cream (REF) was formulated with MDG (0.3 g/100 g). For the sake of  
152 comparison and based on suppliers' suggestions, the emulsifier substitutes were all tested at  
153 the same concentration (1.8 g/100 g), with the exception of WPP (2.0 g/100 g) and ACD (0.35  
154 g/100 g) for which different doses were suggested. The different amounts of emulsifying  
155 ingredients were balanced in the ice cream mix formulations changing the amounts of whole

156 milk and skim milk powder in order to have a constant content of fat ( $4.1 \pm 0.1$  g/100 g),  
157 sugars ( $21.0 \pm 0.4$  g/100 g), and total solids ( $30.7 \pm 0.1$  g/100 g). Moreover, in order to better  
158 understand the role of emulsifiers on the ice cream properties, a mix formulation with neither  
159 MDG nor any emulsifier substitute was developed under the same constraints (named NE).

160

#### 161 *2.4. Ice cream production*

162 Ice cream mixes were prepared as reported in Moriano and Alamprese (2017). Briefly,  
163 pasteurization was carried out up to 85 °C for 1 min on a 15 kg batch by using a Pastomaster  
164 60 Tronic (Carpigiani S.r.l, Anzola Emilia, Italy). After 24-hour aging at 4 °C, the mix was  
165 frozen with a Labotronic 20-30 batch freezer (Carpigiani S.r.l, Anzola Emilia, Italy),  
166 recording extrusion time with a timer. After the product temperature measurement, ice cream  
167 samples were packaged and stored for 24 h at -30 °C. A conditioning phase at -16 °C for 24 h  
168 was carried out before analyses. Two production replicates were carried out for each  
169 formulation.

170

#### 171 *2.5. Ice cream mix analyses*

172 After aging, the ice cream mixes of each production were evaluated for density (g/mL;  $n = 3$ ),  
173 soluble solids (°Bx;  $n = 5$ ), and rheological behavior ( $n = 2$ ) as reported by Moriano and  
174 Alamprese (2017). Moreover, the theoretical freezing point depression (FPD) was calculated  
175 according to Whelan, Vega, Kerry, and Goff (2008), considering technical sheet data of all  
176 the ingredients. As for rheological behavior, flow curves were measured at 4 °C in a 100-500  
177  $s^{-1}$  range of shear rate, by using a Physica MCR 300 rheometer (Anton Paar, Graz, Austria)  
178 equipped with coaxial cylinders (CC27). Results are expressed in terms of apparent viscosity  
179 (mPa·s) at  $290 s^{-1}$ , consistency coefficient  $K$  (mPa·s<sup>n</sup>), and flow behavior index  $n$   
180 (dimensionless).

181

## 182 *2.6. Ice cream analyses*

183 For each production, overrun (%; n = 10), firmness (N; n = 20), and melting behavior (n = 3)  
184 of ice cream samples were determined as reported in Alamprese, Foschino, Rossi, Pompei,  
185 and Savani (2002), using 225 mL samples for overrun and melting and 60 mL samples for  
186 firmness. Briefly, firmness was measured by a penetration test carried out at ambient  
187 temperature with a dynamometer (4301, Instron Ltd., High Wycombe, UK) equipped with a  
188 100 N load cell and a stainless-steel probe (8 mm diameter). Melting behavior was evaluated  
189 in a cabinet at  $20 \pm 1$  °C for 90 min and expressed in terms of starting time (min) and rate  
190 (g/min), calculated from the linear part of the melting curve. During melting also the ice  
191 cream shape retention indexes  $R_t/R_0$  were determined, by applying the image analysis method  
192 reported by Moriano and Alamprese (2017).  $R_t/R_0$  (dimensionless) represents the ratio  
193 between the height and the width of the ice cream sample ( $R_t$ ) calculated at different times  
194 (15, 30, 45, 60, 75, 90 min) and referred to the initial ratio  $R_0$ .

195

## 196 *2.7. Statistical analyses*

197 One-way analysis of variance (ANOVA) was applied to the analytical data in order to find out  
198 significant effects of the emulsifier type. The Least Significant Difference (LSD) test ( $P <$   
199  $0.05$ ) was used to calculate significant differences within the average data obtained in the  
200 characterization of the emulsifier substitutes, or between properties of an ice cream sample  
201 and REF. In this latter case, the results of the two ice cream production replicates carried out  
202 for each formulation were compared.

203 The Pearson product moment correlations between each pair of variables and the relative  
204 significance were calculated in order to measure the strength of the linear relationship  
205 between the analyzed parameters.

206 Statistical analyses were carried out by using the Statgraphics Centurion 18 software  
207 (Statgraphics Technology Inc., The Plains, VI).

208

### 209 **3. Results and discussion**

#### 210 *3.1. Mono- and di-glycerides of fatty acids*

211 MDG, the most common ice cream emulsifiers, showed the second-best value of EAI after  
212 WPP; all the other tested products had significantly lower emulsifying activity (Table 2). This  
213 result highlights the ability of MDG in favoring the formation of emulsions. In fact, as  
214 explained by Pearce & Kinsella (1978), EAI is a measure of the interfacial area stabilized per  
215 unit weight of emulsifier. However, the ability of MDG in stabilizing emulsions against  
216 creaming (CS) was not so high and similar to that of WPC, WPP, and SPI. Indeed, no  
217 significant correlation was found between EAI and CS (Table 3). To the best of our  
218 knowledge, no other published studies tested EAI and CS of MDG, thus no comparisons can  
219 be made.

220 Due to their surface activity at the air/serum interface (Goff & Hartel, 2013), MDG were able  
221 to increase the air incorporation in REF mix, thus lowering the density with respect to the  
222 formulation NE that did not contain any kind of emulsifier (Table 4). The average soluble  
223 solid content of all the ice cream mixes was  $32.3 \pm 1.6$  °Bx, according to the rationale of the  
224 recipe development based on keeping constant total solid and sugar contents. Calculation of  
225 FPD based on theoretical composition of the different mixes revealed a very similar freezing  
226 temperature (ranging from -2.42 to -2.52 °C) for all the samples, according to the constant  
227 total solid and sugar content of the developed formulations. The limited amount of the  
228 emulsifier substitutes, despite their different salt content (technical sheet data), did not  
229 significantly affect FPD, thus assuring soundness of the ice cream quality comparisons. Great  
230 differences were observed for the rheological behavior of the mixes. All the samples

231 significantly differed from REF for the apparent viscosity, and almost all for K and n. In  
232 particular, the absence of emulsifiers (sample NE) significantly decreased apparent viscosity  
233 and K, while increasing n, meaning that the mix was more fluid and less structured than the  
234 REF one. This result indicated a mix-structuring effect of MDG, probably related to their  
235 already explained surface activity at the air/serum interface, favoring air entrapment.  
236 Moreover, MDG can promote fat nucleation during mix aging, thus structuring fat by partial  
237 crystallization (Goff & Hartel, 2013).  
238 Melting behavior of NE clearly highlighted a specific role of MDG on this ice cream property  
239 (Table 5). The presence of MDG made the final product (REF) more resistant to melting,  
240 showing a significantly higher starting time ( $t_s$ ) and lower melting rate. Most of the evaluated  
241 emulsifier substitutes exerted a similar or even higher effect, demonstrating their suitability in  
242 substituting MDG. All the samples well maintained their shape during melting, at least until  
243 60 min (Fig. 1). The slower melting of REF with respect to NE resulted in a higher  $R_t/R_0$  at 75  
244 min, when NE was completely melted.

245

### 246 *3.2. Whey protein concentrate*

247 WPC had a protein content significantly higher than that of LPC and WPP, but lower than the  
248 other protein-based emulsifier substitutes (Table 2). A quite low EAI was observed, meaning  
249 a poor ability of WPC to form an emulsion. However, CS of WPC was not significantly  
250 different from that of MDG. Apart from RPC, all the protein-based emulsifier substitutes  
251 behaved similarly in stabilizing emulsions. This can be related to their water holding capacity,  
252 making them thickening agents. In fact, compared to the use of MDG (REF sample), WPC  
253 significantly increased apparent viscosity (+113 %) and K (+214 %) of ice cream mix (Table  
254 4), while reducing the flow behavior index (-12 %). The use of a thickening agent is  
255 recommended to reduce creaming phenomena in emulsions (McClements, 2016).

256 As for ice cream properties, the only significant effect of WPC was on firmness, which  
257 increased with respect to REF, despite the similar overrun and FPD value (Table 5). Similar  
258 results were found also for SPI\_F and PPC\_F; further investigations including measurements  
259 of fat destabilization will be necessary in order to better understand the effect of these  
260 emulsifier substitutes on the ice cream structure.

261

### 262 *3.3. Whey protein concentrate with high phospholipid content*

263 WPP showed the significantly highest EAI value (Table 2), confirming the high emulsifying  
264 capacity of phospholipids from milk globule membranes because of their polar and nonpolar  
265 composition reported by Levin, Burrington, and Hartel (2016a). However, it should be noted  
266 that the authors, analyzing different types of whey protein phospholipid concentrate,  
267 concluded that the functionality of these products may be influenced by several factors,  
268 including starting whey composition, processing conditions, and the amount of denatured  
269 proteins. An intermediate value of CS was measured for WPP, similar to that of most of the  
270 other tested ingredients.

271 No significant differences were observed between REF and WPP\_F mixes, except for a  
272 higher value of apparent viscosity (+10 %) and  $n$  (+7 %) when WPP was used (Table 4).

273 WPP\_F ice cream showed an overrun significantly lower than that of REF (Table 5). This  
274 result was unexpected considering the well-known high foamability of whey proteins (Luck et  
275 al., 2013) and the high EAI measured for this ingredient, which should have indicated a high  
276 surface activity and a good air bubble dispersion inside the ice cream. However, no significant  
277 correlation was found between EAI and overrun (Table 3), confirming the singular role of  
278 emulsifiers in the ice cream structuring, linked to fat destabilization rather than the common  
279 surface activity important in emulsion formation and stabilization. A possible explanation for  
280 the low overrun of WPP\_F is the presence of the high amount of fat in WPP, which can

281 disrupt the viscoelastic protein layer at the air/serum interfaces by reducing the protein-  
282 protein interactions necessary for air bubble stabilization (Levin et al., 2016a). After 90 min  
283 of melting, only five samples out ten were uncompleted melted; among these, WPP\_F showed  
284 the best shape retention index ( $> 0.65$ ), together with LPC\_F.

285

### 286 *3.4. Soy protein isolate*

287 SPI had the significantly highest value of protein content, because it was the only protein  
288 isolate (Table 2). All the other protein-based substitutes were protein concentrates obtained  
289 from different sources. However, SPI did not show the highest EAI and CS values. In fact, no  
290 significant correlations were found between protein content and EAI or CS (Table 3),  
291 highlighting the importance of the protein type and quality over the amount. The possible  
292 presence of other surface-active components in the ingredients and the production method  
293 could also have affected this result. For instance, Karaca et al. (2011) reported similar  
294 emulsifying activities for soy protein and pea protein isolates produced in the same way,  
295 while in this study SPI and PPC showed significantly different EAI values, being an isolate  
296 and a concentrate, respectively, obtained from two different suppliers.

297 With respect to MDG, the use of SPI imparted a significantly higher apparent viscosity (+43  
298 %) and consistency coefficient (+152 %) to the ice cream mix (Table 4), while decreasing the  
299 flow behavior index (-12 %). This resulted in a significantly shorter extrusion time and higher  
300 extrusion temperature (Table 5). Actually, significant correlations were found between these  
301 variables (Table 3). Extrusion time is linked to the initial consistency of the mix because it is  
302 signaled by the freezer when a given energy input is required to maintain a constant speed of  
303 the internal dasher. Thus, if a more viscous mix is loaded in the freezer, it will take a shorter  
304 time to reach the consistency necessary for the extrusion, and the final product will have a

305 higher temperature. As already stated, the higher firmness of SPI\_F ice cream with respect to  
306 REF needs for further investigations.

307

### 308 *3.5. Pea protein concentrate*

309 PPC showed an EAI significantly lower than that of MDG (-49 %), but a higher CS (+5 %)  
310 (Table 2).

311 Similarly to the other protein-based emulsifier substitutes, PPC significantly affected the ice  
312 cream mix rheological behavior compared to MDG (Table 4), with an increase in apparent  
313 viscosity and K (+37 % and +501%, respectively), and a decrease in n (-36 %).

314 PPC\_F ice cream showed a firmness significantly higher than that of REF (+207%), with a  
315 lower melting rate (-11 %) (Table 5). In this case too, further investigations are needed to  
316 better understand the real role of PPC on ice cream structure.

317

### 318 *3.6. Lupine protein concentrate*

319 LPC showed the significantly lowest protein content, coupled with the lowest value of EAI  
320 (Table 2).

321 LPC\_F mix had a significantly lower density with respect to REF (Table 4), possibly due to  
322 the good foaming capacity of the protein concentrate used, which can favor air incorporation  
323 when ice cream mix is stirred during pasteurization, cooling, and, to a lesser extent, aging.

324 According to Pozani, Doxastakis, and Kiosseoglou (2002), foaming properties of lupine  
325 proteins may improve or deteriorate as a result of the heat treatment applied during  
326 production, depending on molecular unfolding or aggregation.

327 Like most of the other emulsifier substitutes, LPC significantly increased apparent viscosity  
328 (+77 %) and K (+124 %) of the mix with respect to the use of MDG (Table 4).

329 The LPC\_F ice cream showed an overrun significantly lower than that of REF (Table 5).  
330 However, the low overrun could be only apparent due to the low mix density value (used in  
331 the calculation of overrun). Actually, considering the same density of REF, the overrun of  
332 LPC\_F would have been  $49.2 \pm 3.6\%$ , not significantly different from the REF one. The use  
333 of LPC significantly increased  $t_s$  and decreased melting rate with respect to REF, also  
334 conferring the best shape retention index ( $\geq 0.65$ ), like WPP (Fig. 1).

335

### 336 *3.7. Rice protein concentrate*

337 RPC showed the best emulsifying capacity among the plant protein-based ingredients and the  
338 highest CS (Table 2). This is in contrast with the results by Zhao, Shen, Wu, Zhang, and Xu  
339 (2020), who reported a very low EAI for rice proteins in comparison with soybean and pea  
340 proteins, probably due to the very low solubility of their rice protein concentrate at pH 7. In  
341 fact, Mun, Shin, and Kim (2016) demonstrated that solubility of rice proteins depends on the  
342 rice cultivar used for the extraction and emulsifying properties are affected not only by  
343 protein solubility but also by other properties, such as the amino acid composition and the  
344 hydrophilic-lipophilic balance.

345 RPC\_F mix showed a density value significantly higher than that of REF (Table 4). Thus, it is  
346 presumable that the used rice proteins had low foaming capacity, as observed also by Zhao et  
347 al. (2020) for a different commercial product, resulting in a denser ice cream mix. The  
348 significantly lower value of Brix degree was probably due to the interference of some RPC  
349 components during the analysis with the digital refractometer. The increase in apparent  
350 viscosity (+19 %) and K (+446 %) was coupled with the highest decrease in  $n$  (-40 %).  
351 As for the ice cream characteristics, only melting rate was significantly decreased (-11 %)  
352 with the use of RPC instead of MDG (Table 5).

353

354 *3.8. Citrus fibers*

355 CF showed EAI values significantly higher than those of the legume protein-based  
356 ingredients, but lower than that of MDG (Table 2). CF surface properties are mainly linked to  
357 protein residues within the pectin and their covalent bonding (Wallecan et al., 2015).  
358 Emulsion stabilizing properties of CF were very low, resulting in the significantly lowest CS  
359 value, despite the high water retention capacity of orange pulp fiber found in a previous work  
360 by Wallecan et al. (2015), ascribed to cellulose and ensuring the thickening of the water  
361 phase. As demonstrated by Chatsisvili, Amvrosiadis, and Kiosseoglou (2012), it is possible  
362 that the progressive water absorption by pulp particles during emulsion storage forced the oil  
363 droplets to interact more intensively, compressing against each other and thus enhancing the  
364 possibility to coalesce.

365 Like RPC\_F, also CF\_F showed significantly lower values of soluble solid content with  
366 respect to REF (Table 4), probably due to interferences during the measurement. The use of  
367 CF resulted in extremely high values of apparent viscosity and K, which impaired a good  
368 management of the mix, due to difficulties in pouring the sample, and affected freezing  
369 behavior. In fact, CF\_F showed the significantly lowest extrusion time and highest extrusion  
370 temperature (Table 5). These results are related to the high water holding capacity of CF. As a  
371 consequence, CF\_F ice cream showed an overrun significantly lower than that of REF.  
372 Besides the lower whipping time in the freezer, the very high apparent viscosity of the mix  
373 could have also decreased the mix whipping rate due to difficulties in air penetration (Goff &  
374 Hartel, 2013). A significant effect of the high water-retention of CF was observed also on the  
375 ice cream melting behavior. CF\_F showed the highest  $t_s$  and the lowest melting rate values.  
376 Indeed, mix apparent viscosity and K resulted to be highly correlated ( $P < 0.001$ ) with both  $t_s$   
377 and rate (Table 3) as reported also by Wu, Freire, and Hartel (2019). However, during melting

378 CF\_F showed the worst retention indexes (Fig. 1), even if a significant difference compared  
379 to REF was observed only at 30 min.

380

### 381 *3.9. $\alpha$ -cyclodextrin*

382 ACD showed an EAI significantly higher than those of the legume protein-based ingredients,  
383 but lower than that of MDG (Table 2). Its emulsifying properties are linked to the peculiar  
384 molecular structure:  $\alpha$ -cyclodextrin is a cyclic oligosaccharide composed of six  $\alpha$ -D-  
385 glucopyranose units resulting in a shallow truncated cone shape with a hydrophilic annulus  
386 and a largely hydrophobic cavity (Leclercq & Nardello-Rataj, 2016). CS value of ACD was  
387 the second best among all the emulsifier substitutes. These good properties of ACD could be  
388 ascribed to the formation of a highly stable Pickering emulsion, as already reported by  
389 Leclercq and Nardello-Rataj (2016) who demonstrated the ability of cyclodextrin to self-  
390 assemble into colloidal particles, partially wettable by water and oil.

391 Contrarily to most of the studied emulsifier substitutes, the use of ACD significantly  
392 decreased apparent viscosity (-34 %) and K (-47 %), while increasing n (+7 %), meaning that  
393 the mix was more fluid and less structured than the REF one (Table 4).

394 No significant effects of ACD on ice cream characteristics were observed (Table 5, Fig. 1),  
395 meaning that the use of  $\alpha$ -cyclodextrin allowed the production of a final product with the  
396 same quality of REF.

397

### 398 **Conclusions**

399 The study evaluated different commercial ingredients that can be used as MDG substitutes in  
400 ice cream formulation, highlighting their different properties. In particular, results showed  
401 that the beneficial effects of MDG in ice cream are mainly related to an increase in mix  
402 viscosity and a slowdown of ice cream melting behavior. Apart from  $\alpha$ -cyclodextrin, all the

403 other tested ingredients increased the mix apparent viscosity and K, giving sometimes too  
404 high values not compatible with a proper behavior of the mixes in the production plant  
405 (sample CF\_F). All the emulsifier substitutes resulted in good melting properties of ice  
406 creams, comparable with those of REF or even better. In particular, LPC and CF improved  
407 both the melting rate and starting time, whereas PPC and RPC had a significant effect only on  
408 the rate. However, it is important to underline that the source and process conditions of the  
409 different substitutes can affect their technological properties. Thus, the results of this work  
410 can provide a first guide for ice cream producers, but a careful evaluation of the several  
411 products present on the market is absolutely necessary in order to make a correct choice and a  
412 proper balance of the ice cream formulation.

413 In conclusion, the study demonstrated that at the tested doses there was not a single ingredient  
414 that could be considered as effective as MDG on ice cream properties. Probably a  
415 combination of different substitutes in different amounts can be a valuable strategy in order to  
416 effectively substitute MDG and attain the goal of an ice cream with good quality and clean  
417 label. In this perspective, a Design of Experiment approach to the study of different  
418 substitutes' mixing can be a valid tool for an efficient optimization of the ice cream  
419 formulation.

420

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428

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516

517 **Figure legends**

518 **Fig. 1.** Shape retention index ( $R_t/R_0$ ) of the ice cream samples as a function of melting time;  
519 a) REF, ●; NE, ●; WPC\_F, ■, WPP\_F, ◆; SPI\_F, ▲; b) PPC\_F, ○; LPC\_F, ○; RPC\_F, □,  
520 CF\_F, ◇; ACD\_F, △. Error bars represent standard deviation values of two production  
521 replicates. REF: reference ice cream formulation containing mono- and di-glycerides of fatty  
522 acids; NE: ice cream formulation without emulsifiers; WPC\_F, ice cream formulation with  
523 whey protein concentrate; WPP\_F, ice cream formulation with whey protein concentrate at  
524 high phospholipid content; SPI\_F, ice cream formulation with soy protein isolate; PPC\_F, ice  
525 cream formulation with pea protein concentrate; LPC\_F, ice cream formulation with lupine  
526 protein concentrate; RPC\_F, ice cream formulation with rice protein concentrate; CF\_F, ice  
527 cream formulation with citrus fibers; ACD\_F, ice cream formulation with  $\beta$ -cyclodextrin.

**Table 1.** Formulation (g/100 g) of the ice cream samples prepared to study the effect of different commercial emulsifier substitutes.

Ingredient	Ice cream samples									
	REF	NE	WPC_F	WPP_F	SPI_F	PPC_F	LPC_F	RPC_F	CF_F	ACD_F
Pasteurized whole milk	76.0	76.3	76.3	76.7	76.3	76.3	76.3	76.3	76.3	76.55
Pasteurized milk cream	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.1
Skim milk powder	5.4	5.4	3.6	3.0	3.6	3.6	3.6	3.6	3.6	4.8
Sucrose	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Dextrose	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Stabilizers	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
MDG	0.3	-	-	-	-	-	-	-	-	-
WPC	-	-	1.8	-	-	-	-	-	-	-
WPP	-	-	-	2.0	-	-	-	-	-	-
SPI	-	-	-	-	1.8	-	-	-	-	-
PPC	-	-	-	-	-	1.8	-	-	-	-
LPC	-	-	-	-	-	-	1.8	-	-	-
RPC	-	-	-	-	-	-	-	1.8	-	-
CF	-	-	-	-	-	-	-	-	1.8	-
ACD	-	-	-	-	-	-	-	-	-	0.35

MDG, mono- and di-glycerides of fatty acids; WPC, whey protein concentrate; WPP, whey protein concentrate with high phospholipid content; SPI, soy protein isolate; PPC, pea protein concentrate; LPC, lupine protein concentrate; RPC, rice protein concentrate; CF, citrus fibers; ACD,  $\alpha$ -cyclodextrin.

**Table 2.** Values (mean  $\pm$  standard deviation) of protein content, emulsifying activity index (EAI) and creaming stability (CS) of mono- and di-glycerides of fatty acids and ingredients used as emulsifier substitutes in ice cream formulation.

Sample	Protein (g/100 g)	EAI (m <sup>2</sup> /g)	CS (%)
MDG	n.d.	311 $\pm$ 4 <sup>h</sup>	69.5 $\pm$ 1.0 <sup>b</sup>
WPC	74.1 $\pm$ 0.8 <sup>c</sup>	140 $\pm$ 3 <sup>b</sup>	71.7 $\pm$ 0.4 <sup>bc</sup>
WPP	66.6 $\pm$ 1.1 <sup>b</sup>	430 $\pm$ 9 <sup>i</sup>	72.0 $\pm$ 0.1 <sup>bc</sup>
SPI	89.3 $\pm$ 1.7 <sup>e</sup>	172 $\pm$ 1 <sup>d</sup>	71.3 $\pm$ 1.5 <sup>bc</sup>
PPC	77.8 $\pm$ 0.4 <sup>d</sup>	157 $\pm$ 3 <sup>c</sup>	73.0 $\pm$ 1.4 <sup>c</sup>
LPC	59.2 $\pm$ 0.3 <sup>a</sup>	107 $\pm$ 1 <sup>a</sup>	72.5 $\pm$ 1.4 <sup>c</sup>
RPC	77.2 $\pm$ 1.4 <sup>d</sup>	246 $\pm$ 7 <sup>g</sup>	82.5 $\pm$ 1.2 <sup>e</sup>
CF	n.d.	210 $\pm$ 3 <sup>e</sup>	62.2 $\pm$ 1.4 <sup>a</sup>
ACD	n.d.	220 $\pm$ 5 <sup>f</sup>	79.4 $\pm$ 0.3 <sup>d</sup>

MDG, mono- and di-glycerides of fatty acids; WPC, whey protein concentrate; WPP, whey protein concentrate with high phospholipid content; SPI, soy protein isolate; PPC, pea protein concentrate; LPC, lupine protein concentrate; RPC, rice protein concentrate; CF, citrus fibers; ACD,  $\alpha$ -cyclodextrin.

<sup>a-i</sup>, mean values in each column followed by different letters were significantly different ( $P < 0.05$ ).

n.d., not determined.

**Table 3.** Pearson product moments correlation between each pair of variables tested on emulsifier substitutes, ice cream mixes and final ice creams. Significant moments ( $P < 0.05$ ) are highlighted in bold.

	Protein	EAI	CS	Density	Sol. solids	FPD	Viscosity	K	n	Extr. time	Extr. temp.	Overrun	Firmness	ts	Rate
Protein	<b>1.000</b>														
EAI	-0.123	<b>1.000</b>													
CS	0.053	0.018	<b>1.000</b>												
Density	0.634	0.512	0.428	<b>1.000</b>											
Sol. solids	0.245	-0.194	-0.345	-0.026	<b>1.000</b>										
FPD	-0.169	0.121	0.137	-0.106	-0.363	<b>1.000</b>									
Viscosity	-0.165	-0.246	<b>-0.739</b>	-0.494	-0.277	0.241	<b>1.000</b>								
K	0.365	-0.276	-0.443	-0.301	-0.430	0.324	<b>0.860</b>	<b>1.000</b>							
n	-0.459	0.395	-0.144	0.060	0.245	-0.398	-0.320	<b>-0.708</b>	<b>1.000</b>						
Extr. time	-0.421	0.276	0.643	0.399	0.072	-0.463	<b>-0.777</b>	<b>-0.767</b>	0.420	<b>1.000</b>					
Extr. temp.	0.283	0.023	<b>-0.674</b>	-0.282	-0.131	0.454	<b>0.688</b>	0.585	-0.122	<b>-0.916</b>	<b>1.000</b>				
Overrun	0.800	0.200	0.410	<b>0.814</b>	0.293	-0.188	-0.489	-0.210	-0.266	0.350	-0.418	<b>1.000</b>			
Firmness	0.648	-0.354	-0.181	0.039	0.436	0.246	-0.034	0.074	-0.276	-0.475	0.418	0.155	<b>1.000</b>		
t <sub>s</sub>	-0.657	-0.082	-0.665	-0.448	-0.498	0.285	<b>0.933</b>	<b>0.819</b>	-0.214	<b>-0.754</b>	<b>0.765</b>	-0.611	-0.065	<b>1.000</b>	
Rate	0.169	0.241	0.545	0.519	0.492	-0.358	<b>-0.918</b>	<b>-0.943</b>	0.529	<b>0.779</b>	<b>-0.668</b>	0.494	0.026	<b>-0.927</b>	<b>1.000</b>

EAI, emulsifying activity index; CS, creaming stability; Sol. solids, soluble solids; FPD, theoretical freezing point depression; K, consistency coefficient; n, flow behavior index; Extr. time, extrusion time; Extr. temp., extrusion temperature; t<sub>s</sub>, starting time of melting; Rate, melting rate.

**Table 4.** Properties of ice cream mixes (mean  $\pm$  s.d. values of two technological replicates) produced with different types of emulsifier substitutes.

Sample	Density (g/mL)	Soluble solids (°Bx)	FPD (°C)	Apparent viscosity (mPa s)	K (mPa s <sup>n</sup> )	n (-)
REF	1.02 $\pm$ 0.01	33.8 $\pm$ 0.1	-2.49	89 $\pm$ 1	575 $\pm$ 40	0.659 $\pm$ 0.006
NE	1.10 $\pm$ 0.01*	33.9 $\pm$ 1.7	-2.48	59 $\pm$ 1*	348 $\pm$ 24*	0.697 $\pm$ 0.001*
WPC_F	1.03 $\pm$ 0.01	33.5 $\pm$ 1.6	-2.39	190 $\pm$ 4*	1810 $\pm$ 432*	0.570 $\pm$ 0.092*
WPP_F	1.07 $\pm$ 0.05	31.9 $\pm$ 2.1	-2.33	98 $\pm$ 3*	472 $\pm$ 6	0.718 $\pm$ 0.003*
SPI_F	1.04 $\pm$ 0.03	33.9 $\pm$ 0.9	-2.39	128 $\pm$ 5*	1453 $\pm$ 12*	0.573 $\pm$ 0.007*
PPC_F	1.03 $\pm$ 0.01	32.7 $\pm$ 0.4	-2.39	122 $\pm$ 4*	3458 $\pm$ 671*	0.410 $\pm$ 0.045*
LPC_F	0.83 $\pm$ 0.03*	32.8 $\pm$ 1.4	-2.40	158 $\pm$ 8*	1290 $\pm$ 133*	0.634 $\pm$ 0.023
RPC_F	1.07 $\pm$ 0.01*	30.2 $\pm$ 1.0*	-2.36	106 $\pm$ 5*	3141 $\pm$ 386*	0.386 $\pm$ 0.009*
CF_F	0.95 $\pm$ 0.03	30.5 $\pm$ 0.1*	-2.40	435 $\pm$ 3*	6609 $\pm$ 198*	0.520 $\pm$ 0.007*
ACD_F	1.07 $\pm$ 0.06	30.1 $\pm$ 1.3	-2.44	58 $\pm$ 4*	300 $\pm$ 16*	0.711 $\pm$ 0.003*

FPD, theoretical freezing point depression; K, consistency coefficient; n, flow behavior index.

REF: reference ice cream formulation containing mono- and di-glycerides of fatty acids; NE: ice cream formulation without emulsifiers; WPC\_F, ice cream formulation with whey protein concentrate; WPP\_F, ice cream formulation with whey protein concentrate at high phospholipid content; SPI\_F, ice cream formulation with soy protein isolate; PPC\_F, ice cream formulation with pea protein concentrate; LPC\_F, ice cream formulation with lupine protein concentrate; RPC\_F, ice cream formulation with rice protein concentrate; CF\_F, ice cream formulation with citrus fibers; ACD\_F, ice cream formulation with  $\alpha$ -cyclodextrin.

\* Result significantly different ( $P < 0.05$ ) from the reference (REF).

**Table 5.** Extrusion parameters and physical properties of ice cream samples (mean  $\pm$  s.d. values of two technological replicates).

Sample	Extrusion time (min)	Extrusion temperature (°C)	Overrun (%)	Firmness (N)	Melting behavior	
					t <sub>s</sub> (min)	rate (g/min)
REF	7.3 $\pm$ 0.1	-8.1 $\pm$ 0.3	50.8 $\pm$ 0.4	17.7 $\pm$ 1.9	16.9 $\pm$ 0.9	2.62 $\pm$ 0.05
NE	6.9 $\pm$ 0.3	-7.8 $\pm$ 0.2	58.5 $\pm$ 0.9	21.5 $\pm$ 1.8	13.4 $\pm$ 0.7*	2.81 $\pm$ 0.02*
WPC_F	6.8 $\pm$ 0.3	-7.9 $\pm$ 0.1	49.9 $\pm$ 0.4	28.8 $\pm$ 0.2*	20.2 $\pm$ 2.5	2.53 $\pm$ 0.19
WPP_F	6.6 $\pm$ 0.1*	-6.8 $\pm$ 0.4	41.6 $\pm$ 0.9*	25.1 $\pm$ 3.7	23.1 $\pm$ 2.4	2.58 $\pm$ 0.01
SPI_F	6.1 $\pm$ 0.2*	-6.5 $\pm$ 0.2*	52.0 $\pm$ 1.4	57.2 $\pm$ 0.8*	21.0 $\pm$ 1.7	2.51 $\pm$ 0.05
PPC_F	6.2 $\pm$ 0.5	-7.2 $\pm$ 0.5	50.6 $\pm$ 1.9	54.4 $\pm$ 3.3*	21.8 $\pm$ 1.1	2.33 $\pm$ 0.01*
LPC_F	6.5 $\pm$ 0.2	-7.4 $\pm$ 0.1	21.6 $\pm$ 0.8*	23.1 $\pm$ 2.8	24.0 $\pm$ 0.5*	2.33 $\pm$ 0.01*
RPC_F	6.9 $\pm$ 0.2	-8.0 $\pm$ 0.1	59.2 $\pm$ 0.2	12.3 $\pm$ 1.1	19.1 $\pm$ 1.3	2.32 $\pm$ 0.03*
CF_F	5.5 $\pm$ 0.1*	-5.9 $\pm$ 0.1*	30.3 $\pm$ 0.8*	21.3 $\pm$ 0.5	50.8 $\pm$ 1.0*	1.60 $\pm$ 0.12*
ACD_F	7.3 $\pm$ 0.1	-7.9 $\pm$ 0.1	38.5 $\pm$ 4.8	20.3 $\pm$ 4.6	22.1 $\pm$ 2.2	2.63 $\pm$ 0.04

t<sub>s</sub>, starting time of melting

REF: reference ice cream formulation containing mono- and di-glycerides of fatty acids; NE: ice cream formulation without emulsifiers;

WPC\_F, ice cream formulation with whey protein concentrate; WPP\_F, ice cream formulation with whey protein concentrate at high

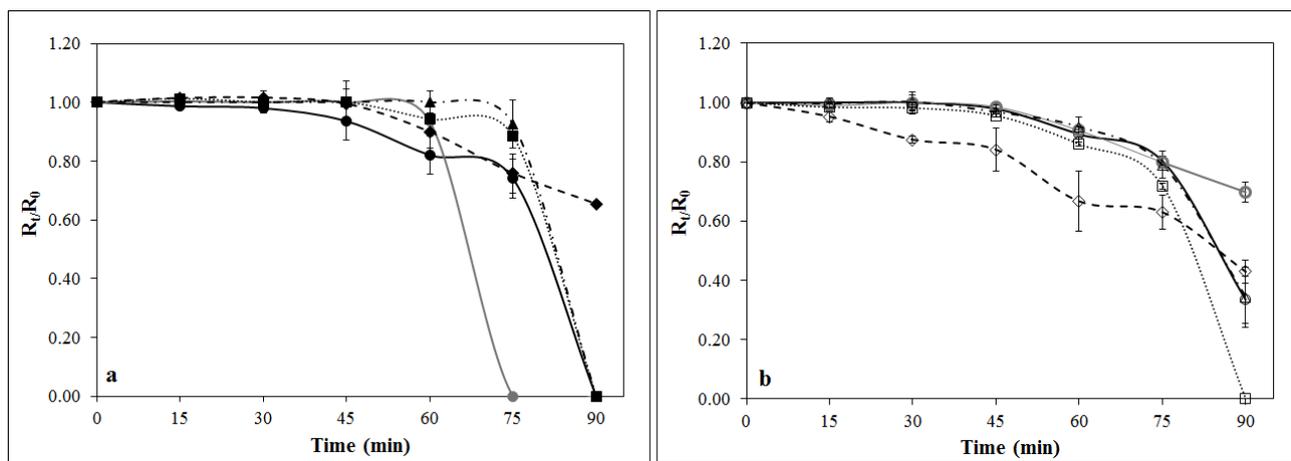
phospholipid content; SPI\_F, ice cream formulation with soy protein isolate; PPC\_F, ice cream formulation with pea protein concentrate;

LPC\_F, ice cream formulation with lupine protein concentrate; RPC\_F, ice cream formulation with rice protein concentrate; CF\_F, ice cream

formulation with citrus fibers; ACD\_F, ice cream formulation with  $\alpha$ -cyclodextrin.

\* Result significantly different ( $P < 0.05$ ) from the reference (REF).

Figure 1.



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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: