Title	Influence of low molecular weight surfactants on the stability of model infant formula emulsions based on hydrolyzed rice protein
Authors	Amagliani, Luca;O'Regan, Jonathan;Kelly, Alan L.;O'Mahony, James A.
Publication date	2022
Original Citation	Amagliani, L., O'Regan, J., Kelly, A. L. and O'Mahony, J. A. (2022) 'Influence of low molecular weight surfactants on the stability of model infant formula emulsions based on hydrolyzed rice protein', LWT - Food Science and Technology, 154, 112544 (9pp). doi: 10.1016/j.lwt.2021.112544
Type of publication	Article (peer-reviewed)
Link to publisher's version	10.1016/j.lwt.2021.112544
Rights	© 2021, The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). - https://creativecommons.org/licenses/by/4.0/
Download date	2026-09-22 16:59:08
Item downloaded from	https://hdl.handle.net/10468/13483

LWT - Food Science and Technology 154 (2022) 112544

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Influence of low molecular weight surfactants on the stability of model infant formula emulsions based on hydrolyzed rice protein

Luca Amagliani [a,b], Jonathan O'Regan [c], Alan L. Kelly [a], James A. O'Mahony [a,*]

[a] School of Food and Nutritional Sciences, University College Cork, Cork, Ireland
[b] Department of Chemistry, Nestle Institute of Material Sciences, Nestlé Research, Lausanne, Switzerland
[c] Nestlé Development Center, Wyeth Nutritionals Ireland, Askeaton, Co. Limerick, Ireland

ARTICLE INFO

Keywords:
Rice protein hydrolyzate
Infant formula
Emulsion
Surfactants

ABSTRACT

The emulsifying properties of rice protein ingredients, and their behaviour in the presence of other non-protein emulsifiers, are essential to support food applications. The objective of this study was to investigate the influence of different low molecular weight surfactants (LMWS) on the stability of model infant formula emulsions (10 g protein L^{-1}, 35 g soybean oil L^{-1}, 50 g carbohydrate L^{-1}) based on a rice protein hydrolyzate (RPH). Inclusion of CITREM and DATEM, at concentrations greater than 1 g L^{-1}, gave emulsions with mean fat globule diameters (D [4,3]) less than 1 μm with good creaming stability, while lecithin was less effective across the concentration range tested (i.e., 1–3 g L^{-1}). Coalescence of the control emulsion was observed upon both storage (10 d at 4 °C) and heat treatment (95 °C × 5 min). Increasing CITREM concentration increased storage and heat stability of the emulsions, while addition of CITREM at 2 g L^{-1} facilitated the formation of a very stable product. This study provides a first insight into the potential of hydrolyzed rice protein to be used as a value-added ingredient in the production of nutritional beverages such as infant formula emulsions, and the influence of LMWS on the stability of such products.

1. Introduction

Proteins are commonly used as food emulsifiers due to their ability to facilitate the formation, and improve the stability, of oil-in-water (O/W) emulsions by forming interfacial films that generate electrostatic (depending on the solvent conditions) and steric (depending on the protein) repulsive forces and barriers between fat globules (Lam & Nickerson, 2013; McClements, 2004; Ozturk & McClements, 2016). The stability of protein-stabilized O/W emulsions is influenced by the different solution conditions (pH, ionic strength, surfactants, biopolymers) and environmental factors (thermal processing, chilling, freezing, drying, homogenization and mechanical agitation) that they experience during their production, storage and utilization (Drapala et al., 2018; McClements, 2004).

The proteins most widely used as emulsifiers in the food industry are those derived from bovine milk, i.e., caseins and whey protein (Wilde, 2009). However, in recent years, the food industry and research communities have shown increased interest in the identification of plant proteins to be used as substitutes to those derived from animal sources in new food formulations, such as vegetarian and vegan products. Increasing the use of plant proteins for human food is also proposed to enhance food security and environmental sustainability (Amagliani et al., 2017b; Day, 2013).

Among the potential plant sources of proteins to be used as functional ingredients in food formulations, rice represents an interesting option. Although rice has a relatively low protein concentration (6–7% in milled rice) when compared with the other major cereals (i.e., wheat, corn and barley), the availability of rice protein is potentially high since the annual production of milled rice worldwide is about 490 million metric tons (USDA, 2018). Rice endosperm and rice bran proteins have been reported to have true digestibility (90.8–94.8%) comparable to that of soy, casein and whey proteins (91.7–94.8%) and, among these ingredients, their biological value (66.7–72.6%) is second only to that of whey protein (78.8%) (Han et al., 2015). Also, rice protein is generally regarded as hypoallergenic (Helm & Burks, 1996) and thus represents a suitable candidate for the development of infant formulas targeted for infants with cow's milk allergy, as a potential alternative to those based on soy proteins or extensively hydrolyzed dairy proteins (Amagliani et al., 2017b).

However, the poor solubility in water of intact rice protein

* Corresponding author.
E-mail address: sa.omahony@ucc.ie (J.A. O'Mahony).

https://doi.org/10.1016/j.lwt.2021.112544
Received 10 June 2020; Received in revised form 30 June 2021; Accepted 27 September 2021
Available online 29 September 2021

ingredients at or around neutral pH, due to the extensive aggregation and crosslinking through disulphide bonds of glutelin (i.e., the main rice protein fraction), considerably limits their range of potential food applications. Hydrolyzed rice protein ingredients typically display better solubility and interfacial properties than their intact counterparts, making them the preferred choice for the manufacture of liquid and semi-liquid foods (Amagliani et al., 2017b, 2019).

Low molecular weight surfactants (LMWS) are also widely used in the food industry for their contribution to formation and stabilization of O/W emulsions. They are typically classified according to their hydrophilic-lipophilic balance (HLB), a measure of the degree of hydrophilicity or hydrophobicity of an emulsifier (Griffin, 1949). LMWS commonly used in food formulations include mono- and diglycerides, acid esters of mono- and diglycerides, such as citric acid esters of mono- and diglycerides of fatty acids (CITREM) and mono- and diacetyl tartaric acid esters of mono- and diglycerides of fatty acids (DATEM), and lecithins. Mono- and diglycerides are non-ionic surfactants, possessing a lipophilic character and thus having low HLB values (3–6) (Moonen & Bas, 2004). CITREM and DATEM are anionic surfactants, both being more hydrophilic, and thus having higher HLB values, compared to the mono- and diglycerides from which they are derived (Gaupp & Adams, 2004a, 2004b). Lecithins are zwitterionic surfactants, with intermediate HLB values of ~8 (McClements, 2005), which can be obtained from animal (e.g., egg yolk) and vegetable (e.g., soybean, rapeseed and sunflower oil) sources (Bueschelberger, 2004).

The use of non-protein emulsifiers such as LMWS is often necessary to stabilize O/W emulsions containing hydrolyzed proteins, peptides or free amino acids, such as infant formulas (McSweeney, 2008). When compared with amphiphilic biopolymers (e.g., proteins), many LMWS are able to stabilize smaller fat globules as they adsorb more rapidly at the oil-water interface during homogenization, reduce the interfacial tension, and are able to stabilize emulsions at lower concentrations (Piorkowski & McClements, 2014). When used in combination with proteins, LMWS can influence the stability of emulsions in several ways, i.e., (i) by competing with proteins for adsorption at the interface, where the most surface-active and/or abundant molecules prevail; (ii) by displacing proteins from the interface due to the higher surface activity of LMWS; (iii) by complexing with proteins, which may lead to an increase or a decrease in the surface activity of the LMWS-protein complex; or (iv) by interacting with proteins at the interface, which may result in a more efficient packing or the disruption of protein-protein interactions within the interfacial film (Nylander et al., 2008).

The purpose of this study was to investigate the influence of different LMWS (i.e., CITREM, DATEM and lecithin), at different levels of addition, on the formation and processing stability of model infant formula emulsions based on hydrolyzed rice protein.

2. Materials and methods

2.1. Materials

The protein ingredient used to prepare the model infant formula emulsions was a commercially available rice endosperm protein hydrolyzate (referred to hereafter as RPH) and was characterized as detailed in Section 2.2. Maltodextrin (Maldex 170, with dextrose equivalent value of 17) was obtained from Syral Belgium NV (Aalst, Belgium). CITREM (Grindsted®) was obtained from Danisco (Copenhagen, Denmark). DATEM (Admul™) was obtained from Kerry Group (Tralee, Co. Kerry, Ireland). De-oiled soybean lecithin (Ultralec® P) was obtained from ADM (Decatur, IL, USA). Soybean oil was obtained from Frylite (Cork, Co. Cork, Ireland).

2.2. Characterization of the rice protein hydrolyzate ingredient

The macronutrient composition, degree of hydrolysis and molecular size distribution of peptides in RPH were analyzed using the methods described by Amagliani at al. (2017a) and are summarized in Table 1.

2.3. Preparation of model infant formula emulsions

The model infant formula emulsions (10 g protein L^{-1}, 35 g oil L^{-1}, 50 g carbohydrate L^{-1}) were prepared as follows: RPH was solubilized in deionised water, which had been preheated to 75 °C, under continuous mixing using an overhead stirrer operating at 500 rpm for 1 h. Once RPH was fully solubilized, maltodextrin was added, the dispersion was allowed to gradually cool to 22 °C while stirring at 500 rpm for 2 h, and its pH was adjusted to 6.8 using 0.1 and 1 N NaOH before being stored at 4 °C for 18 h to facilitate complete rehydration. After this, the dispersion was allowed to re-equilibrate at 22 °C for 1 h; innate carbohydrate levels of RPH were considered when formulating the emulsions. CITREM and DATEM, when used, were dissolved in this aqueous phase at 55 °C, whereas lecithin, when used, was dispersed in the oil phase at 65 °C. LMWS were added at concentrations ranging from 1 to 3 g L^{-1} of the final emulsions. Aqueous and oil phases were subsequently mixed and the blend tempered at 55 °C until homogenization. Preparation of the emulsions involved pre-homogenization using an Ultra-Turrax at 10,000 rpm for 2 min followed by homogenization (double pass) using a two-stage valve homogenizer (APV GEA Niro Soavi S.p.A., Italy) with first and second stage pressures of 15 and 3 MPa, respectively. Following homogenization, the pH of each emulsion was measured and re-adjusted to 6.8 using 0.1 and 1 N NaOH, if needed.

2.4. Measurement of fat globule size distribution

Analysis of the fat globule size distribution (FGSD) of the model infant formula emulsions was performed using a laser light diffraction unit (Mastersizer S, Malvern Instruments Ltd., Worcestershire, UK) equipped with a 300 RF (reverse Fourier) lens and He–Ne laser (λ of 633 nm) as described by Drapala et al. (2015). Measurements were performed on fresh emulsions within 2 h following homogenization, as well as during 10 d of storage at 4 °C and following heat treatment (95 °C for 5 min) for the storage and heat stability studies, respectively (see Section 2.6). Results were calculated using the Mie theory and presented as: D[4,3] (volume-weighted mean particle diameter), D[3,2] (surface-weighted mean particle diameter), D(v,0.5) (particle size below which 50% of sample volume is found) and D(v,0.9) (particle size below which 90% of sample volume is found).

2.5. Accelerated creaming stability

Accelerated creaming stability analysis of the model infant formula

Table 1
Macronutrient composition, degree of hydrolysis (DH) and peptide size distribution of the rice endosperm protein hydrolyzate (RPH) used to prepare the model infant formula emulsions.

RPH composition (%)	
Protein	75.6 ± 0.08
Carbohydrate	13.7 ± 0.20
Total starch	7.45 ± 0.82
Fibre	0.30 ± 0.15
Fat	0.26 ± 0.07
Ash	6.16 ± 0.07
Moisture	4.32 ± 0.06
DH	17.8 ± 0.41
Peptide distribution	**% of total protein**
Insoluble	0.00 ± 0.00
>20 kDa	0.38 ± 0.30
10–20 kDa	0.08 ± 0.01
5–10 kDa	0.67 ± 0.03
2–5 kDa	6.25 ± 0.03
1–2 kDa	14.9 ± 0.07
0.5–1 kDa	25.6 ± 0.09
<0.5 kDa	52.1 ± 0.09

emulsions was performed using a LUMiSizer (L.U.M. GmbH, Berlin, Germany), a multi-sample analytical centrifuge which employs the STEP-Technology® (Space- and Time-resolved Extinction Profiles) to measure the intensity of near-infrared light transmitted over the entire length of a sample as a function of time and position. The emulsions, contained in screw-capped glass tubes, were inverted three times and aliquots (0.4 mL) were placed in polycarbonate measurement cells and centrifuged at 1,500 rpm (262–327 *g*, depending on the distance from the rotor within the length of the measurement cells) for 7.5 h, as described by Murphy et al. (2011). The creaming rate was expressed as the slope ($R^2 = 0.98$) of the integral transmission-time graphs created using data provided by the Sepview 5.1 (L.U.M. GmbH, Berlin, Germany) software.

2.6. Storage and heat stability

Storage and heat stability of the model infant formula emulsions were investigated using CITREM as LMWS at concentrations ranging from 0.5 to 2 g L^{-1} of the final emulsions. The emulsions were prepared as described in Section 2.3 and sodium azide (0.05%, w/v) was added to the emulsions following homogenization in order to prevent microbial growth during storage. Storage stability was investigated by measuring FGSD of the emulsions, as described in Section 2.4, after 1, 3, 7 and 10 d of storage at 4 °C. For the assessment of heat stability, the emulsions, 1 day post preparation, were subjected to heat treatment using an AR-G2 controlled stress rheometer (TA Instruments, Crawley, UK) fitted with a starch-pasting cell. The cell had an internal diameter of 36 mm, the impeller had a diameter of 32 mm, and the operating gap between the cell and the impeller at the base of the geometry was 0.55 mm. Aliquots of the emulsions (28 mL) were loaded into the starch pasting cell and tempered for 5 min at 15 °C. After the conditioning step, samples were heated from 15 to 95 °C at a rate of 10 °C min^{-1}, held at 95 °C for 5 min, cooled from 95 to 15 °C at a rate of 10 °C min^{-1} and held for 5 min at 15 °C. The shear rate was fixed at 15 s^{-1} and viscosity data were collected at 5 s intervals. FGSD was measured before and after heat treatment as described in Section 2.4.

2.7. Confocal laser scanning microscopy analysis

The visualization of emulsions was performed using an Olympus FluoView® FV1000 confocal laser scanning microscope (CLSM) (Olympus Corporation, Tokyo, Japan). Protein and oil were fluorescently labelled by adding 50 μL of 0.05% (w/v) Nile blue A (Sigma-Aldrich, St. Louis, MO, US) into 1 mL of emulsion followed by vortex mixing. The samples (10 μL) were placed between a microscope slide and a coverslip and imaging of the emulsions was performed at excitation wavelengths of 488 nm and 633 nm provided by Ar and He/Ne lasers, respectively. Representative images (512 × 512 pixels in size) of each sample were taken using a 60x oil immersion objective with a numerical aperture of 1.4.

2.8. Statistical data analysis

All model infant formula emulsions were produced in three independent trials on separate days and all measurements were carried out in triplicate. Analysis of variance (ANOVA; Tukey's HSD test) was carried out using Minitab® 16 (Minitab Ltd, Coventry, UK) statistical analysis package. The level of statistical significance was determined at $P < 0.05$.

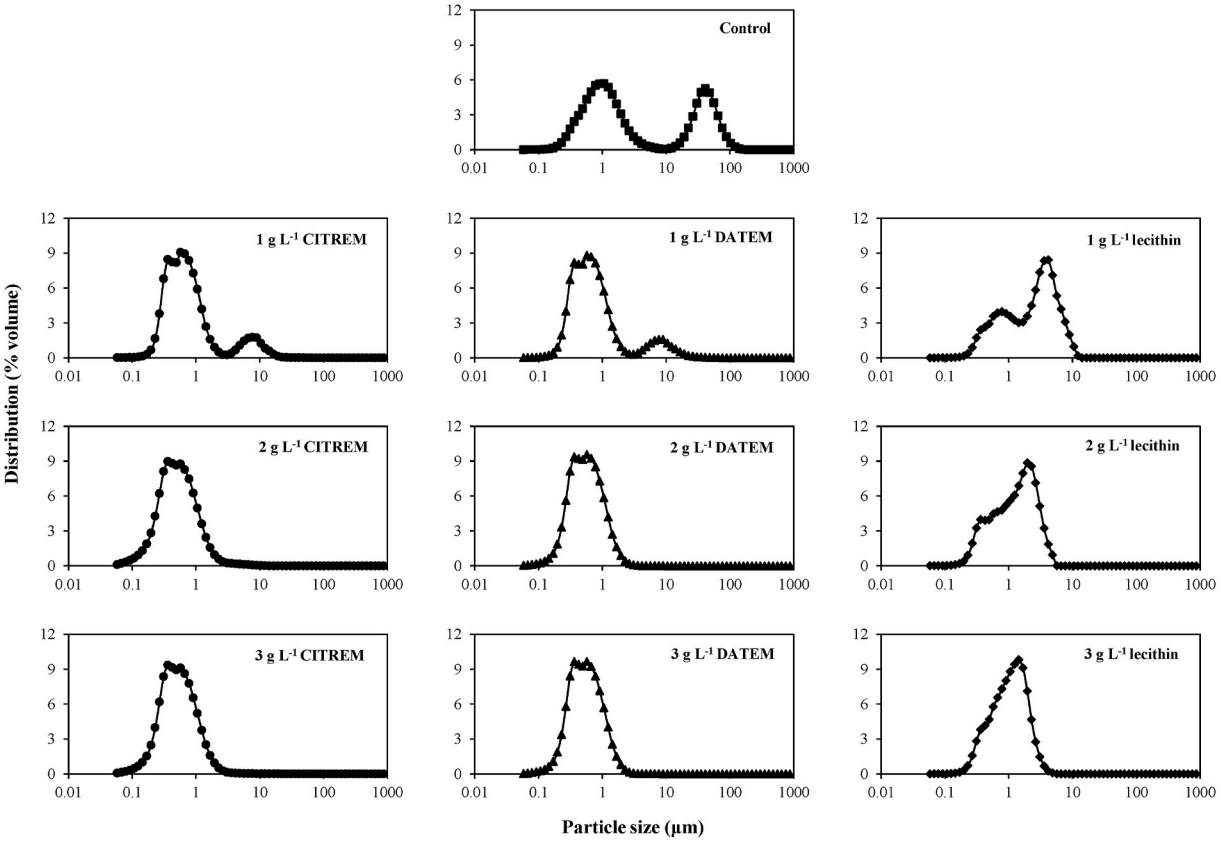

Fig. 1. Fat globule size distributions of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and of the RPH-based emulsions containing CITREM, DATEM or lecithin at concentrations ranging from 1 to 3 g L^{-1}, as measured upon homogenization.

3. Results and discussion

3.1. Influence of low molecular weight surfactants on the production and creaming stability of RPH-based model infant formula emulsions

The FGSD profiles, and associated parameters, upon homogenization of the control and LMWS-containing emulsions are shown in Fig. 1 and Table 2, respectively. RPH displayed poor emulsifying properties, as the emulsion prepared using this protein ingredient alone had a bimodal FGSD, with the second peak in the size range 10–100 μm (Fig. 1); the control emulsion had a D[4,3] of 16.1 μm and a D(v,0.9) of 50.0 μm (Table 2). Food emulsions are generally considered to be of good quality when characterized by a narrow and monomodal particle size distribution, with a D[4,3] of less than 1 μm (Drapala et al., 2015). The high proportion of low molecular weight (MW) peptides, with more than 90% of the peptides having MW $\leq$ 2 kDa (Table 1), may explain the poor emulsifying capacity of RPH.

The emulsifying properties of proteins from various sources have been shown to improve as a result of limited enzymatic hydrolysis due to the decreased MW, increased solubility, exposure of hydrophobic groups previously buried within the protein core and higher flexibility of the resulting peptides, leading to faster migration and stronger anchoring to the oil-water interface, and the formation of more viscoelastic interfacial layers (Amagliani et al., 2017b; Lam & Nickerson, 2013; Tamm et al., 2016). On the other hand, extensive enzymatic hydrolysis is known to impair the emulsifying properties of proteins, as the high proportion of small peptides and free amino acids generated tends to saturate the continuous phase rather than adsorbing to the oil-water interface. Although RPH was completely soluble in water (Table 1), its poor emulsifying properties may be due to the small peptides characterizing this ingredient having insufficient hydrophobicity to adsorb to the oil-water interface, or to such peptides forming interfacial layers characterized by low viscoelasticity, thus being unable to properly stabilize the fat globules (Lam & Nickerson, 2013).

With the inclusion of LMWS in the RPH-based formulation, the FGSD was considerably changed, with a shift towards a monomodal distribution being observed with increasing LMWS concentrations (Fig. 1). Emulsions with narrow FGSD, in the range 0.1–1 μm, were obtained when CITREM or DATEM were added at concentrations greater than 1 g L^{-1}, while the FGSD was broader when lecithin was added at the same concentrations (Fig. 1). D[4,3] values generally decreased with increasing LMWS concentration, although increasing the concentration of CITREM or DATEM from 2 to 3 g L^{-1} caused only a marginal decrease in D[4,3] (Table 2). CITREM and DATEM were significantly ($P < 0.05$) more effective compared to lecithin in reducing the average fat globule size of the RPH-based emulsions across the concentration range tested; D[4,3] and D(v,0.9) values were in the range 0.58–0.61 μm and 1.05–1.09 μm, respectively, when CITREM or DATEM were added to the emulsions at concentrations greater than 1 g L^{-1} (Table 2).

The results obtained from accelerated creaming stability testing provided a similar trend to the FGSD results, the control emulsion being particularly unstable, with the slope (R^2 = 0.98) of the integral transmission-time graph showing a value of 65.4; however, the slope decreased considerably to values in the ranges 11.5–41.8, 8.23–25.9 and 7.79–19.8 on addition of LMWS at concentrations of 1, 2 and 3 g L^{-1}, respectively, with CITREM and DATEM being significantly ($P < 0.05$) more effective than lecithin in stabilizing the emulsions across the concentration range tested (Table 2). The improvement in terms of stability provided by the LMWS to the RPH-based emulsions was also clearly noticeable from the normalized transmission-position graphs, which show a reduction in transmission at high radial positions and in the spacing between the transmission profiles with increasing levels of LMWS (Fig. 2), indicative of greater emulsion stability.

Lecithin is commonly used in the production of commercial infant formula emulsions based on intact proteins, mainly to improve emulsion stability during processing and storage *via* a combination of electrostatic and van der Waals forces, protein displacement, and the formation of protein/phospholipid complexes, resulting in a reduction in interfacial tension and fat globule size (McSweeney, 2008). In the study of McSweeney et al. (2008), lecithin was shown to improve the heat stability of a model infant formula emulsion based on intact milk proteins. However, Drapala et al. (2015) reported that, although addition of lecithin (10–50 g L^{-1} of oil) to a model infant formula emulsion (15.5 g protein L^{-1}, 35 g oil L^{-1}, 70 g carbohydrate L^{-1}) stabilized with partially hydrolyzed whey protein (DH = 10.7%) decreased fat globule size upon homogenization, the inclusion of lecithin at levels of 10–30 g L^{-1} of oil promoted coalescence during storage.

In the current study, CITREM and DATEM proved to be more effective than lecithin in reducing the fat globule size of the emulsions upon homogenization and in stabilizing the emulsions towards creaming. This may be ascribed to the high HLB values of CITREM and DATEM, which make them particularly suitable for use in O/W emulsions. These LMWS are indeed commonly used to stabilize commercial infant formula emulsions formulated using extensively hydrolyzed proteins, peptides and amino acids (McSweeney, 2008). McSweeney (2007) reported that addition of CITREM (9 g L^{-1}) to a model infant formula emulsion containing hydrolyzed whey protein (DH = 25–32%) facilitated the formation of smaller fat globules and provided stability towards creaming and coalescence. In the same study, inclusion of DATEM (0.4 g L^{-1}) reduced the D[4,3] value of the emulsion from 0.90 to 0.61 μm; the emulsion containing DATEM creamed rapidly upon centrifugation, but was stable towards coalescence, as its fat globule size distribution was relatively unchanged after 12 weeks of storage at 4 °C.

Table 2
Fat globule size distribution parameters and creaming rate of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and the RPH-based emulsions containing CITREM, DATEM or lecithin at concentrations ranging from 1 to 3 g L^{-1}.

	Concentration (g L^{-1})	D[4,3]	D[3,2]	D(v,0.5)	D(v,0.9)	Creaming rate (–)
Control	No added LMWS	16.1 ± 0.48^{a}	1.09 ± 0.06^{b}	1.53 ± 0.17^{b}	50.0 ± 2.85^{a}	65.4 ± 4.00^{a}
CITREM	1	1.50 ± 0.36^{cB}	0.54 ± 0.03^{dB}	0.60 ± 0.03^{eB}	4.50 ± 2.25^{bA}	11.8 ± 0.54^{eB}
	2	0.61 ± 0.02^{dB}	0.38 ± 0.01^{eB}	0.47 ± 0.01^{eB}	1.09 ± 0.04^{bB}	8.23 ± 0.15^{eB}
	3	0.59 ± 0.02^{dB}	0.39 ± 0.00^{eB}	0.47 ± 0.02^{eB}	1.06 ± 0.05^{bB}	7.79 ± 0.07^{eB}
DATEM	1	1.66 ± 0.25^{cB}	0.53 ± 0.03^{dB}	0.61 ± 0.04^{eB}	3.79 ± 2.20^{bA}	11.5 ± 0.45^{eB}
	2	0.60 ± 0.02^{dB}	0.42 ± 0.02^{eB}	0.50 ± 0.02^{eB}	1.07 ± 0.05^{bB}	8.81 ± 0.12^{eB}
	3	0.58 ± 0.01^{dB}	0.42 ± 0.01^{eB}	0.49 ± 0.01^{eB}	1.05 ± 0.03^{bB}	8.51 ± 0.03^{eB}
Lecithin	1	2.78 ± 0.13^{bA}	1.19 ± 0.01^{aA}	2.46 ± 0.09^{aA}	5.75 ± 0.36^{bA}	41.8 ± 2.71^{bA}
	2	1.42 ± 0.01^{cA}	0.81 ± 0.01^{cA}	1.25 ± 0.02^{cA}	2.73 ± 0.02^{bA}	25.9 ± 0.71^{cA}
	3	1.10 ± 0.03^{cdA}	0.74 ± 0.02^{cA}	0.98 ± 0.03^{dA}	1.95 ± 0.05^{bA}	19.8 ± 0.70^{dA}

($^{a-e}$) Values followed by different superscript lower-case letters in the same column are significantly different ($P < 0.05$).
($^{A-C}$) For emulsions containing the same LMWS concentration, values followed by different superscript upper-case letters in the same column are significantly different ($P < 0.05$).

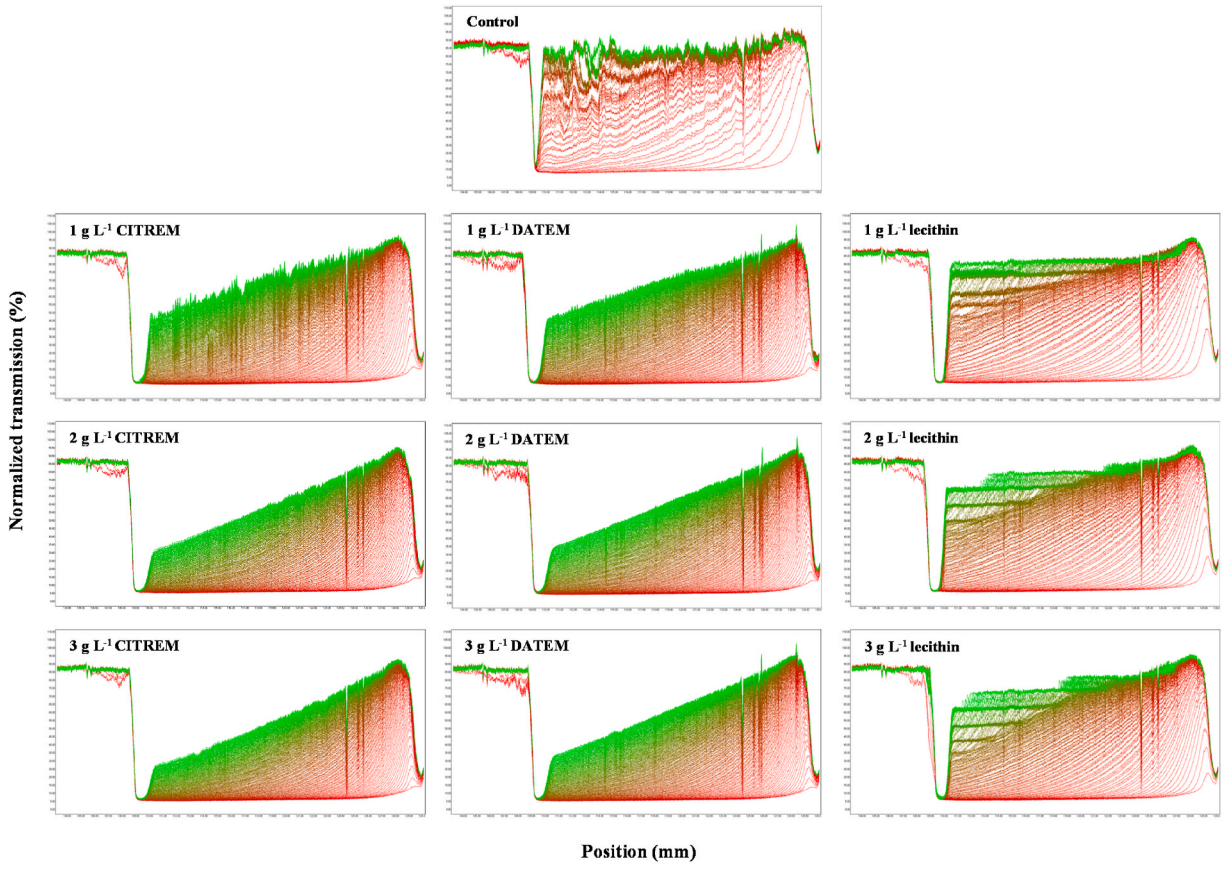

Fig. 2. Representative transmission profiles generated from accelerated creaming stability analysis of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and the RPH-based emulsions containing CITREM, DATEM or lecithin at concentrations ranging from 1 to 3 g L^{-1} during centrifugation at 1,500 rpm for 7.5 h.

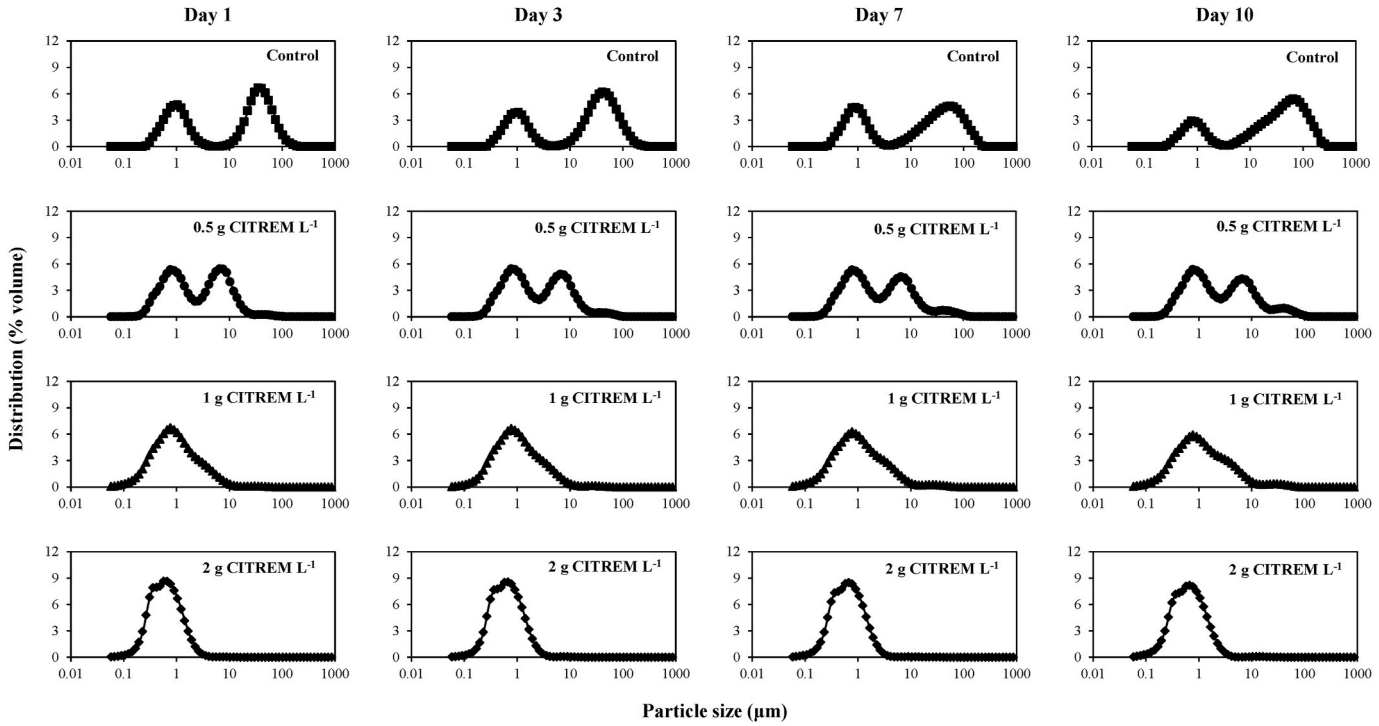

Fig. 3. Fat globule size distribution of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and of the RPH-based emulsions containing CITREM at concentrations ranging from 0.5 to 2 g L^{-1}, as measured after 1, 3, 7 and 10 d of storage at 4 °C.

3.2. Influence of CITREM on the storage and heat stability of RPH-based model infant formula emulsions

The storage and heat stability of RPH-based emulsions prepared using CITREM was studied due to the ability of this LMWS to considerably reduce fat globule size and improve the stability towards creaming of the emulsions. Notable changes were observed in the control emulsion during 10 d of storage at 4 °C, with the FGSD shifting toward higher particle size ranges (Fig. 3). D[4,3] and D(v,0.9) values of the control emulsion reached 41.4 μm and 103 μm after 10 d of storage, indicating considerable destabilization of the fat globules (Table 3).

In addition to significantly reducing the average fat globule size of the RPH-based emulsions on homogenization, CITREM provided stability during storage, with the FGSD of the CITREM-containing emulsions changing only very slightly, if at all, during 10 d of storage at 4 °C (Fig. 3). However, the emulsion containing 0.5 g L^{-1} CITREM exhibited a bimodal FGSD after 1 d of storage, with the second peak having a mean particle diameter at ~10 μm. When CITREM was added at concentrations greater than 0.5 g L^{-1}, a monomodal FGSD was retained throughout the 10 d of storage, and a narrow size distribution was observed for the emulsion containing 2 g L^{-1} CITREM (Fig. 3). In emulsions containing 0.5 or 1 g L^{-1} CITREM, D[4,3] values increased from 4.61 to 6.25 and from 1.62 to 2.46 μm, respectively, after 10 d of storage (Table 3); on the other hand, the D(v,0.9) increased from 10.3 to 14.7 μm in the emulsion containing 0.5 g L^{-1} CITREM, and from 3.40 to 4.72 μm in the emulsion containing 1 g L^{-1} CITREM after 10 d of storage (Table 3).

In order to determine the mechanism of destabilization (i.e., coalescence or flocculation) of the emulsions during storage, a 0.2% (w/v) solution of the dissociating agent sodium dodecyl sulphate (SDS) was used as the dispersant during analysis of FGSD of the samples stored for 10 d, an approach employed in previous studies (Drapala et al., 2015; Tirok et al., 2001). The FGSD remained unchanged for the control sample on adding SDS, indicating that coalescence of fat globules was the main cause of instability in this emulsion, while flocculation was the main mechanism of destabilization in emulsions containing 0.5 or 1 g L^{-1} CITREM, as indicated by significant ($P < 0.05$) decreases in both D[4,3] and D(v,0.9) on adding SDS (data not shown).

Changes in fat globule size during storage in the emulsion containing 2 g L^{-1} CITREM were marginal (i.e., from 0.71 to 0.80 μm after 10 d of storage) (Table 3). This result was confirmed by confocal laser scanning microscopy (CLSM) analysis, which showed that the fine and uniform distribution of the fat globules observed in the emulsion after 1 d of storage (Fig. 6a) was retained after 10 d of storage (Fig. 6b). According to McSweeney (2007), a whey protein hydrolyzate-based model infant formula emulsion containing CITREM (9 g L^{-1}) was stable towards coalescence, as shown by the unchanged FGSD after 12 weeks of storage at 4 °C. These results indicate that CITREM provided electrostatic and/or steric stability to the RPH-based emulsions, a phenomenon which may be ascribed to displacement by CITREM of RPH peptides adsorbed at the oil-water interface and/or interactions between CITREM and peptides in the RPH. Previous work has shown that CITREM has the ability to form complexes with proteins (i.e., legumin and sodium caseinate) in a bulk aqueous medium, mainly through hydrophobic interactions (Antipova et al., 2001).

The viscosity profiles on heat treatment at 95 °C for 5 min of the emulsions are shown in Fig. 4. The emulsions showed no peaks or major transitions in viscosity with increasing temperature, and they all had similar viscosity values (8.2–8.8 mPa s) during the holding step at 95 °C. However, it is noteworthy that emulsions containing CITREM had higher initial and final viscosity values (15.6–15.9 and 16.2–16.5 mPa s, respectively) compared to the control emulsion (13.5 and 13.6 mPa s, respectively). The smaller fat globule size and the higher viscosity of emulsions containing CITREM explain their greater stability to creaming compared to the control emulsion since, according to Stokes' law, the rate of creaming of emulsions decreases with decreasing diameter of fat globules and with increasing viscosity of the continuous phase (Damodaran, 2005). A higher viscosity of the continuous phase would result in reduced mobility of the fat globules and thus a reduction in their collision frequency. The increased stability to creaming observed with increasing CITREM concentration may therefore be ascribed entirely to differences in fat globule size, as the emulsions containing different CITREM concentrations had similar viscosities.

A decrease in the number of smaller fat globules (i.e., reduction in volume of the first peak) and a substantial increase in the number of larger fat globules (i.e., growth of the second peak) was observed following heat treatment of the control emulsion at 95 °C for 5 min (Fig. 5), which displayed a two-fold increase in D[4,3], i.e., from 24.9 to 49.3 μm (Table 3). Previous studies have reported destabilization, mainly *via* coalescence, of emulsions produced with extensively

Table 3
Fat globule size distribution parameters of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and the RPH-based emulsions containing CITREM at concentrations ranging from 0.5 to 2 g L^{-1} upon storage at 4 °C for 10 d or upon heat treatment at 95 °C for 5 min.

	Storage time (d)	Heat treatment	D[4,3]	D[3,2]	D(v,0.5)	D(v,0.9)
Control	1	No	24.9 ± 1.82^{a}	1.82 ± 0.05^{a}	20.9 ± 0.03^{a}	60.4 ± 5.96^{a}
	1	Yes	49.3 ± 5.02^{a}	4.55 ± 0.29^{a}	46.8 ± 3.96^{a}	93.4 ± 11.2^{a}
	3	No	31.8 ± 6.08^{a}	2.24 ± 0.08^{a}	25.8 ± 3.89^{a}	75.6 ± 15.6^{a}
	7	No	34.2 ± 6.47^{a}	1.95 ± 0.16^{a}	21.8 ± 5.76^{a}	89.8 ± 15.3^{a}
	10	No	41.4 ± 4.12^{a}	2.60 ± 0.17^{a}	29.0 ± 5.05^{a}	103 ± 11.7^{a}
0.5 g L^{-1} CITREM	1	No	4.61 ± 0.59^{bA}	1.16 ± 0.10^{bA}	2.11 ± 0.09^{bA}	10.3 ± 0.96^{bA}
	1	Yes	7.49 ± 1.15^{bA}	1.56 ± 0.16^{bA}	6.96 ± 1.09^{bA}	14.2 ± 2.99^{bA}
	3	No	4.96 ± 0.01^{bA}	1.13 ± 0.11^{bA}	1.82 ± 0.29^{bA}	10.9 ± 0.47^{bA}
	7	No	6.14 ± 0.98^{bA}	1.13 ± 0.10^{bA}	1.87 ± 0.30^{bA}	13.6 ± 3.15^{bA}
	10	No	6.25 ± 0.60^{bA}	1.13 ± 0.10^{bA}	1.83 ± 0.23^{bA}	14.7 ± 3.33^{bA}
1 g L^{-1} CITREM	1	No	1.62 ± 0.08^{bcB}	0.59 ± 0.07^{cB}	0.83 ± 0.06^{cB}	3.40 ± 0.29^{bB}
	1	Yes	5.08 ± 0.36^{bB}	1.14 ± 0.05^{bB}	4.68 ± 0.08^{bB}	10.5 ± 0.93^{bAB}
	3	No	1.70 ± 0.13^{bB}	0.61 ± 0.06^{cB}	0.87 ± 0.05^{bB}	3.55 ± 0.44^{bB}
	7	No	2.03 ± 0.29^{bB}	0.60 ± 0.07^{cB}	0.89 ± 0.06^{bB}	3.98 ± 0.58^{bB}
	10	No	2.46 ± 0.48^{bB}	0.62 ± 0.06^{cB}	0.95 ± 0.08^{bB}	4.72 ± 0.94^{bB}
2 g L^{-1} CITREM	1	No	0.71 ± 0.05^{cB}	0.46 ± 0.02^{cB}	0.57 ± 0.04^{dC}	1.31 ± 0.12^{bC}
	1	Yes	2.07 ± 0.05^{bC}	0.57 ± 0.01^{cC}	0.66 ± 0.01^{bC}	6.80 ± 0.03^{bB}
	3	No	0.74 ± 0.04^{bC}	0.46 ± 0.02^{cB}	0.58 ± 0.03^{bB}	1.35 ± 0.09^{bC}
	7	No	0.77 ± 0.02^{bB}	0.47 ± 0.02^{cB}	0.59 ± 0.04^{bB}	1.38 ± 0.04^{bB}
	10	No	0.80 ± 0.02^{bC}	0.46 ± 0.04^{cB}	0.58 ± 0.04^{bB}	1.44 ± 0.03^{bB}

($^{a-c}$) Values followed by different superscript lower-case letters in the same column and for the same measurement stage are significantly different ($P < 0.05$).
($^{A-C}$) For emulsions containing CITREM, values followed by different superscript upper-case letters in the same column and for the same measurement stage are significantly different ($P < 0.05$).

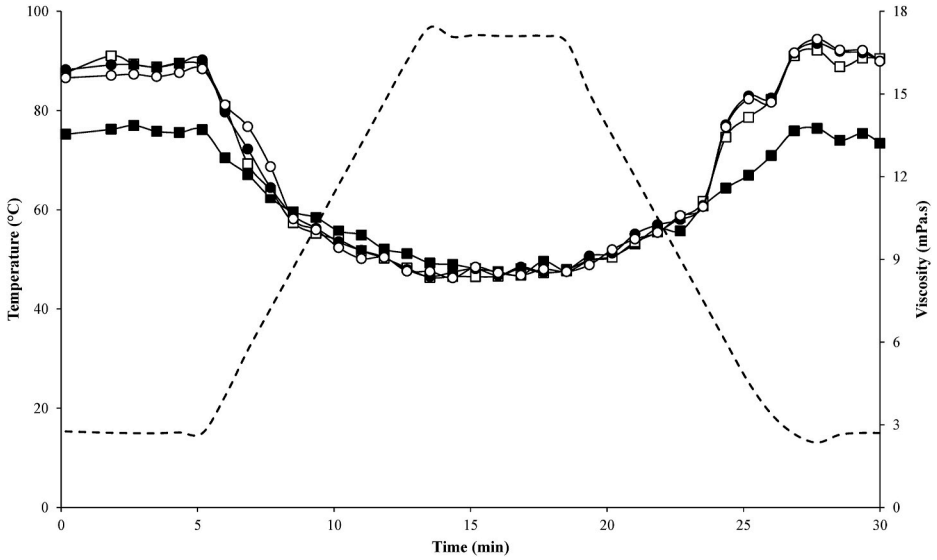

Fig. 4. Viscosity profiles upon heat treatment at 95 °C for 5 min of the control RPH-based model infant formula emulsion (i.e., no added LMWS) (■) and of the RPH-based emulsions containing 0.5 (□), 1 (●) and 2 (○) g L^{-1} CITREM. The dashed line represents the temperature profile.

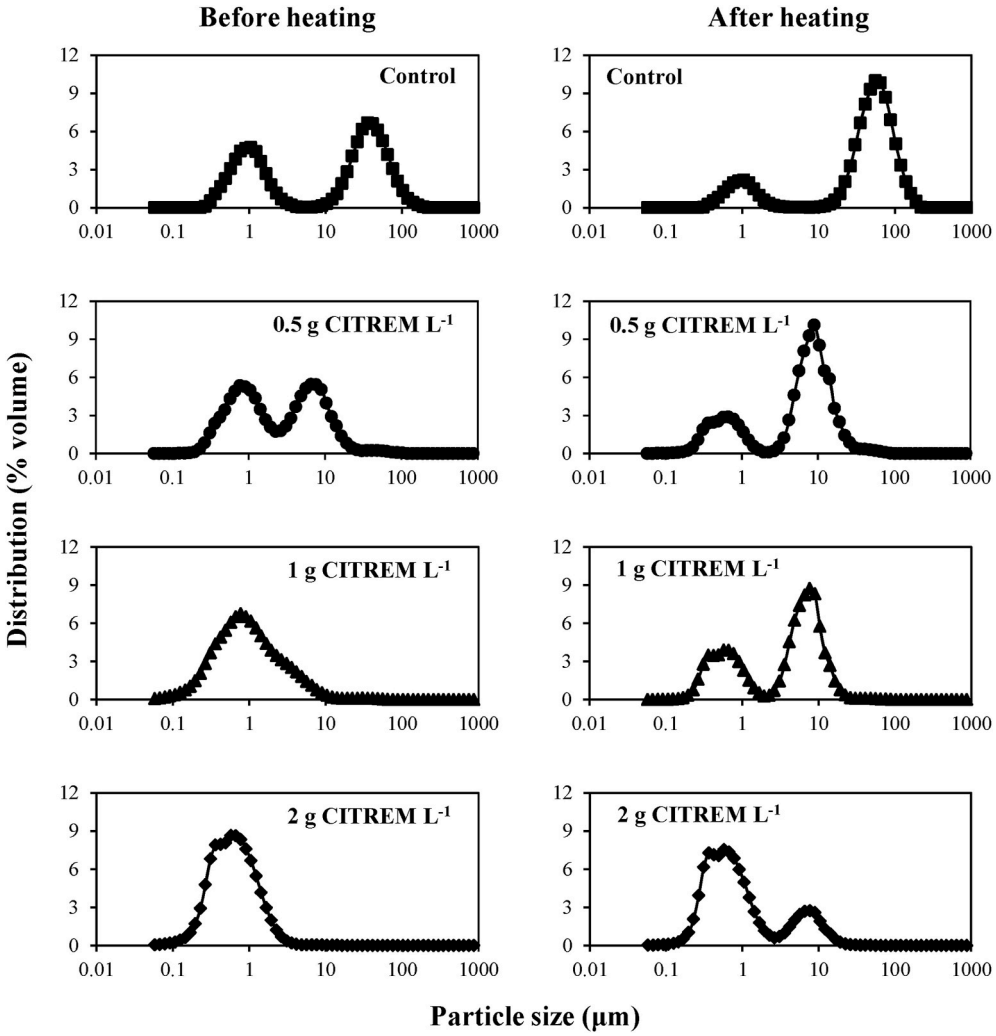

Fig. 5. Fat globule size distribution of the control RPH-based model infant formula emulsion (i.e., no added LMWS) and of the RPH-based emulsions containing CITREM at concentrations ranging from 0.5 to 2 g L^{-1}, measured before and after heat treatment at 95 °C for 5 min.

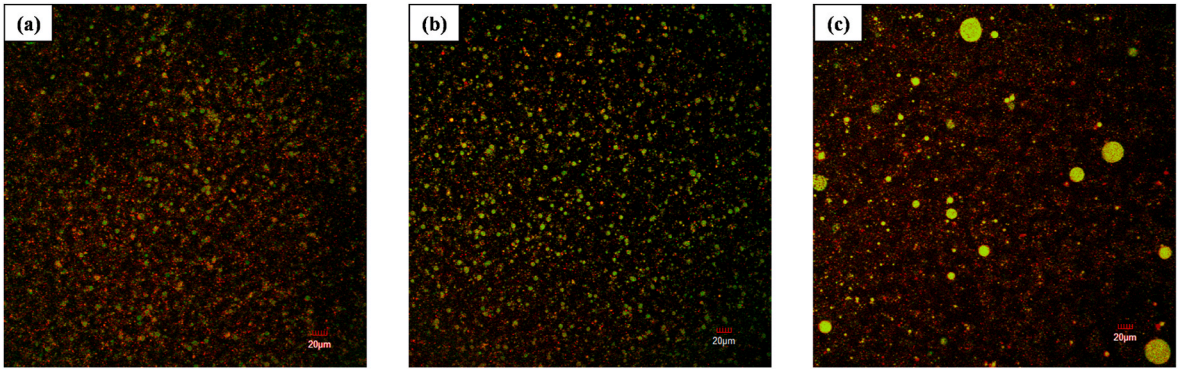

Fig. 6. Confocal laser scanning microscopy images of the RPH-based model infant formula emulsion containing 2 g L^{-1} CITREM after 1 d (a) and 10 d (b) of storage at 4 °C, and following heat treatment at 95 °C for 5 min (c). The oil phase appears green while the protein phase appears red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

hydrolyzed whey protein upon heat treatment, due to the inability of the peptides adsorbed at the oil-water interface to provide sufficient electrostatic or steric stabilization to the fat globules (Agboola et al., 1998; Ye & Singh, 2006).

Emulsions containing CITREM also displayed instability to heat treatment, as shown by changes in their FGSD after being thermally treated at 95 °C for 5 min. The emulsion containing 0.5 g L^{-1} CITREM displayed an increase in the number of larger fat globules, while the FGSD of emulsions containing 1 and 2 g L^{-1} CITREM became bimodal following thermal processing (Fig. 5). As already observed from the storage stability tests, the stability of the emulsions following heat treatment increased with increasing CITREM concentration; D[4,3] values of emulsions containing 0.5, 1 and 2 g L^{-1} CITREM were 7.49, 5.08 and 2.07 μm, respectively, following heat treatment. The destabilizing effect of heat treatment on the emulsion containing 2 g L^{-1} CITREM was further demonstrated using CLSM analysis, showing the presence of some large fat globules (10–20 μm) in this emulsion following heat treatment (Fig. 6c). The main destabilization mechanism upon heating of the control emulsion and the emulsions containing CITREM was coalescence, as their average fat globule size did not change when FGSD analysis was performed using a 0.2% (w/v) SDS solution as dispersant (data not shown).

4. Conclusions

A model infant formula emulsion formed using RPH alone had large sized fat globules and poor stability towards creaming, quiescent storage (10 d at 4 °C) and heat treatment (95 °C for 5 min). However, the inclusion of non-protein emulsifiers along with the RPH significantly reduced the fat globule size and increased the stability towards creaming of the RPH-based emulsions, with CITREM and DATEM being more effective than lecithin. The storage and heat stability of the RPH-based emulsions increased with increasing CITREM concentration (0.5–2 g L^{-1}); a very stable emulsion was formed when CITREM was included at a concentration of 2 g L^{-1}, as shown by the unchanged FGSD after 10 d of storage at 4 °C. Furthermore, increasing CITREM concentration considerably improved heat stability by reducing the propensity for fat globules in the emulsion to coalesce, due to modification of the interfacial layer composition and properties. However, CITREM concentration would need to be increased to generate heat-stable fat globules if using heating conditions more severe than those used in this study (e.g., UHT and in-container sterilization). This study provides a first insight into the potential of hydrolyzed rice protein to be used as a value-added ingredient in the production of nutritional beverages such as infant formula emulsions, and the influence of LMWS on the stability of such products. The relevance of this work is highlighted by the increasing research and commercial interest in the development of food products based on alternative proteins, with a view to addressing challenges such as food security and environmental sustainability.

CRediT authorship contribution statement

Luca Amagliani: Conceptualization, Methodology, Investigation, Visualization, Writing – original draft. **Jonathan O'Regan:** Conceptualization, Project administration, Writing – review & editing. **Alan L. Kelly:** Supervision, Writing – review & editing. **James A. O'Mahony:** Funding acquisition, Conceptualization, Supervision, Writing – review & editing, Project administration.

Declaration of competing interest

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.

Acknowledgements

The authors would like to acknowledge Nestlé for providing financial support for this study. The authors would also like to acknowledge the Biosciences Imaging Centre (Department of Anatomy and Neuroscience, University College Cork, Ireland) for access to the microscopy facilities and Dr Juliana Valle Costa Silva (School of Food and Nutritional Sciences, University College Cork, Ireland) for her assistance in performing confocal laser scanning microscopy analysis.

References

Agboola, S. O., Singh, H., Munro, P. A., Dalgleish, D. G., & Singh, A. M. (1998). Stability of emulsions formed using whey protein hydrolyzate: Effects of lecithin addition and retorting. *Journal of Agricultural and Food Chemistry, 46*, 1814–1819.

Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017a). Composition and protein profile analysis of rice protein ingredients. *Journal of Food Composition and Analysis, 59*, 18–26.

Amagliani, L., O'Regan, J., Kelly, A. L., & O'Mahony, J. A. (2017b). The composition, extraction, functionality and applications of rice proteins: A review. *Trends in Food Science & Technology, 64*, 1–12.

Amagliani, L., O'Regan, J., Schmitt, C., Kelly, A. L., & O'Mahony, J. A. (2019). Characterization of the physicochemical properties of intact and hydrolysed rice protein ingredients. *Journal of Cereal Science, 88*, 16–23.

Antipova, A. S., Semenova, M. G., Belyakova, L. E., & Il'in, M. M. (2001). On relationships between molecular structure, interaction and surface behaviour in mixture: Small-molecule surfactant + protein. *Colloids and Surfaces B: Biointerfaces, 21*, 217–230.

Lecithins Bueschelberger, H. G. (2004). In R. J. Whitehurst (Ed.), *Emulsifiers in food technology* (pp. 1–39). Oxford, UK: Blackwell Publishing Ltd.

Damodaran, S. (2005). Protein stabilisation of emulsions and foams. *Journal of Food Science, 70*, R54–R66.

Day, L. (2013). Proteins from land plants - potential resources for human nutrition and food security. *Trends in Food Science & Technology, 32*, 25–42.

Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2015). Influence of lecithin on the processing stability of model whey protein hydrolysate-based infant formula emulsions. *International Journal of Dairy Technology, 68*, 322–333.

Drapala, K. P., Mulvihill, D. M., & O'Mahony, J. A. (2018). A review of the analytical approaches used for studying the structure, interactions and stability of emulsions in nutritional beverage systems. *Food Structure, 16*, 27–42.

Gaupp, R., & Adams, W. (2004a). Acid esters of mono- and diglycerides. In R. J. Whitehurst (Ed.), *Emulsifiers in food technology* (pp. 59–85). Oxford, UK: Blackwell Publishing Ltd.

Gaupp, R., & Adams, W. (2004b). Di-acetyltartaric esters of monoglycerides (DATEM) and associated emulsifiers in bread making. In R. J. Whitehurst (Ed.), *Emulsifiers in food technology* (pp. 86–109). Oxford, UK: Blackwell Publishing Ltd.

Griffin, W. C. (1949). Classification of surface-active agents by "HLB". *Journal of the Society of Cosmetic Chemists, 1*, 311–326.

Han, S. W., Chee, K. M., & Cho, S. J. (2015). Nutritional quality of rice bran protein in comparison to animal and vegetable protein. *Food Chemistry, 172*, 766–769.

Helm, R. M., & Burks, A. W. (1996). Hypoallergenicity of rice protein. *Cereal Foods World, 41*, 839–843.

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chemistry, 141*, 975–984.

McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science, 9*, 305–313.

McClements, D. J. (2005). Emulsion ingredients. In D. J. McClements (Ed.), *Food emulsions: Principles, practice and techniques* (2nd ed., pp. 95–174). Boca Raton, FL: CRC Press.

McSweeney, S. L. (2007). *Stability of model ready-to-feed infant formula emulsions*. Cork: Ph.D. Thesis, National University of Ireland.

McSweeney, S. L. (2008). Emulsifiers in infant nutritional products. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), *Food emulsifiers and their applications* (2nd ed., pp. 233–261). New York, NY: Springer.

McSweeney, S. L., Healy, R., & Mulvihill, D. M. (2008). Effect of lecithin and monoglycerides on the heat stability of a model infant formula emulsion. *Food Hydrocolloids, 22*, 888–898.

Moonen, H., & Bas, H. (2004). Mono- and diglycerides. In R. J. Whitehurst (Ed.), *Emulsifiers in food technology* (pp. 40–58). Oxford, UK: Blackwell Publishing Ltd.

Murphy, E. G., Tobin, J. T., Roos, Y. H., & Fenelon, M. A. (2011). The effect of high velocity steam injection on the colloidal stability of concentrated emulsions for the manufacture of infant formulations. *Procedia Food Science, 1*, 1309–1315.

Nylander, T., Arnebrant, T., Bos, M., & Wilde, P. (2008). Protein/emulsifier interactions. In G. L. Hasenhuettl, & R. W. Hartel (Eds.), *Food emulsifiers and their applications* (2nd ed., pp. 89–171). New York, NY: Springer.

Ozturk, B., & McClements, D. J. (2016). Progress in natural emulsifiers for utilization in food emulsions. *Current Opinion in Food Science, 7*, 1–6.

Piorkowski, D. T., & McClements, D. J. (2014). Beverage emulsions: Recent developments in formulation, production, and applications. *Food Hydrocolloids, 42*, 5–41.

Tamm, F., Herbst, S., Brodkorb, A., & Drusch, S. (2016). Functional properties of pea protein hydrolysates in emulsions and spray-dried microcapsules. *Food Hydrocolloids, 58*, 204–214.

Tirok, S., Scherze, I., & Muschiolik, G. (2001). Behaviour of formula emulsions containing hydrolysed whey protein and various lecithins. *Colloids and Surfaces B: Biointerfaces, 21*, 149–162.

USDA. (2018). *Grain: World markets and trade. December 2018*. Washington, DC: United States Department of Agriculture, Foreign Agricultural Service.

Wilde, P. J. (2009). Emulsions and nanoemulsions using dairy ingredients. In M. Corredig (Ed.), *Dairy-derived ingredients: Food and nutraceutical uses* (pp. 539–564). Cambridge, UK: Woodhead Publishing.

Ye, A., & Singh, H. (2006). Heat stability of oil-in-water emulsions formed with intact or hydrolysed whey proteins: Influence of polysaccharides. *Food Hydrocolloids, 20*, 269–276.