

Title	The use of inline high-shear rotor-stator mixing for preparation of high-solids milk-protein-stabilised oil-in-water emulsions with different protein:fat ratios
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Publication date	2017-09-27
Original Citation	O'Sullivan, J. J., Drapala, K. P., Kelly, A. L. and O'Mahony, J. A. (2017) 'The use of inline high-shear rotor-stator mixing for preparation of high-solids milk-protein-stabilised oil-in-water emulsions with different protein:fat ratios', Journal of Food Engineering, In Press. doi:10.1016/j.jfoodeng.2017.10.015
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/ S0260877417304466 - 10.1016/j.jfoodeng.2017.10.015
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Download date	2025-04-03 04:38:01
Item downloaded from	https://hdl.handle.net/10468/4999



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Accepted Manuscript

The use of inline high-shear rotor-stator mixing for preparation of high-solids milkprotein-stabilised oil-in-water emulsions with different protein:fat ratios



PII:	S0260-8774(17)30446-6
DOI:	10.1016/j.jfoodeng.2017.10.015
Reference:	JFOE 9048
To appear in:	Journal of Food Engineering
Received Date:	09 August 2017
Revised Date:	17 October 2017
Accepted Date:	22 October 2017

Please cite this article as: Jonathan J. O'Sullivan, Kamil P. Drapala, Alan L. Kelly, James A. O' Mahony, The use of inline high-shear rotor-stator mixing for preparation of high-solids milk-proteinstabilised oil-in-water emulsions with different protein:fat ratios, *Journal of Food Engineering* (2017), doi: 10.1016/j.jfoodeng.2017.10.015

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Highlights

- Emulsification of different fat-filled milk formulations was investigated.
- Emulsification was achieved using novel inline high-shear mixing technology.
- The emulsification process was monitored inline using pressure drop analysis.
- Pressure drop data allowed for the estimation of viscosity during emulsion formation.

1	The use of inline high-shear rotor-stator mixing for preparation of high-solids milk-
2	protein-stabilised oil-in-water emulsions with different protein:fat ratios
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10 Abstract:

The emulsification of refined palm oil (RPO) in a continuous phase consisting of skim milk 11 concentrate (SMC) and maltodextrin with a dextrose equivalent value of 17 (MD17) to produce 12 fat-filled milk emulsions (FFMEs), was studied. A novel inline high-shear mixing (IHSM) 13 method was used to produce emulsions, and three protein contents were investigated at a fixed 14 RPO content of 12%: low (7.7%), medium (10.5%) and high (13%). Pressure drop 15 measurement was used as an inline approach to determine viscosity using the Hagen-Poiseuille 16 equation. In addition, offline viscometry, particle size and emulsion stability analyses were 17 performed. Emulsion fat droplet size decreased significantly (P < 0.05) as a function of number 18 of passes through the IHSM, due to an effective increase in residence time. Furthermore, inline 19 pressure drop data demonstrated that the emulsification process displayed two distinct stages: 20 21 (i) oil injection, and (ii) reduction in fat droplet size, irrespective of protein content.

22

Keywords: High solids emulsions, High-shear inline mixer, Pressure drop, Skim milk
concentrate, Refined palm oil, Maltodextrin

25 1. Introduction

Milk is a highly versatile raw material and, over the past century, significant advances 26 have been achieved in its fractionation into a wide variety of components (Fox, 2008; 27 O'Sullivan & O'Mahony, 2016). These constituent-based ingredients are often recombined, 28 sometimes with ingredients derived from other sources (e.g., plant-derived proteins, 29 carbohydrates and lipids), to achieve different formulations, which can be utilised as final 30 products by the consumer (e.g., enriched milk powders), or be further processed as ingredients 31 by food manufacturers (e.g., protein concentrates/isolates or blends, for example in the 32 manufacture of infant formulae) (O'Connell & Flynn, 2007). One such example is fat-filled 33 milk powders (FFMPs), which are dried protein-stabilised emulsions, typically produced by 34 solids concentration (e.g., by evaporation) and homogenisation followed by spray drying. 35 These systems are intended either for direct reconstitution by consumers, or as ingredients in a 36 variety of recombined applications, such as beverages, ice cream, confectionary and bakery 37 products (Sharma et al., 2012; Vignolles et al., 2007). 38

The formulation of these fat-filled milk emulsions (FFMEs) prior to spray-drying 39 typically involves blending of skim milk concentrate (SMC; *i.e.*, a concentrated protein and 40 lactose solution) with oils (*i.e.*, often derived from plants, such as coconut or palm oils) to 41 achieve the required ratio of protein to fat, and with additional carbohydrates added (Sharma 42 et al., 2012). SMC is produced by removal of the fat from milk through centrifugation and 43 concentrating the remaining stream to a solids content of >35% (w/w) (O'Connell & Flynn, 44 2007). FFMEs are typically prepared by injecting fats into SMC, followed by emulsification 45 using two-stage valve homogenisation. The fats that are used are typically derived from plants 46 47 and are either solid or semi-solid at ambient temperature, in order to be comparable to milk fat. Thus, prior to injection, these fats need to be liquefied and dosed into the SMC at elevated 48 temperatures, in the range 50-60°C usually (Vignolles et al., 2007). The ratio of protein with 49

respect to a fixed fat content is influenced by addition of carbohydrates (which reduces the 50 protein content), often maltodextrins; these are polysaccharides of variable chain length 51 produced by partial hydrolysis of starch, which are defined by their dextrose equivalent (DE) 52 value (Drapala et al., 2016; Mulcahy et al., 2016; O'Mahony et al., 2017). Use of higher 53 concentrations of protein (e.g., >5% w/w) in emulsion systems, in comparison to lower 54 concentrations of protein, yields smaller emulsion droplets which are more resistant to 55 56 emulsion instability, due to greater coverage of the droplet interface, reducing the propensity towards coalescence and increasing the electrostatic repulsive interactions between protein-57 58 stabilised emulsion droplets (O'Sullivan et al., 2014; O'Sullivan, Park, & Beevers, 2016). Furthermore, dairy-derived carbohydrate sources, such as lactose or permeate from 59 ultrafiltration of skim milk, are widely employed to vary the protein content with respect to fat 60 in a similar fashion to maltodextrin addition. Plant-derived oils and maltodextrin ingredients 61 are commonly used owing to their lower overall cost and reduced powder stickiness challenges 62 during spray-drying, respectively (Gonzalez-Perez & Arellano, 2009; Vega & Roos, 2006). 63

After oil injection and emulsification, these formulations are spray-dried to yield FFMP 64 (Sharma et al., 2012). To the authors' knowledge, there are no studies available in the published 65 literature detailing the formation of these high solids emulsion systems, the role of protein-to-66 fat ratio in their formation and stability, and the inline monitoring of this process from fat 67 injection through to formation of the final emulsion. This study aims to investigate the emulsion 68 formation process for high solids emulsions, using an inline high-shear mixer (IHSM) for 69 emulsification, in a recirculation configuration (i.e., semi-continuous), and to assess the 70 suitability of using a pressure drop approach to monitor the process in real-time, from fat 71 injection through to final emulsion formation. 72

High-shear mixers are widely used for emulsification applications and the dissolution
of powders to form homogeneous solutions (Hall *et al.*, 2013; O'Sullivan *et al.*, 2017). The

configuration of these mixers is that of a rotor-stator, and they can be used in an inline 75 configuration for either continuous processing (*i.e.*, single-pass mode) or semi-continuous 76 processing (*i.e.*, multiple-pass mode), and are highly energy efficient (Hall et al., 2011). The 77 shear rate range for high-shear mixers is typically within the range $20,000 - 100,000 \text{ s}^{-1}$. 78 depending on factors such as tip speed, rotor-stator geometry (e.g., single or double screen) 79 and physical properties (e.g., viscosity, presence of particulates, etc.) of the material being 80 processed (Pacek et al., 2007). Pressure drop across a section of pipeline, for a flowing fluid, 81 can be measured using a pair of pressure transducers, separated by a known distance. Pressure 82 83 drop data provides useful information as to how a process is performing in real-time, as the data can be used to calculate a theoretical viscosity value from the Hagen-Poiseuille equation 84 (Douglas et al., 2005; Mihailova et al., 2015). O'Sullivan et al. (2017) demonstrated the 85 suitability of a pressure drop approach for monitoring the induction of dairy powders in real-86 time, observing different aspects of the process, such as initial contact of the powder with water, 87 and the disintegration of powder particles as a function of processing time. 88

The overall objective of this research was to evaluate the suitability of the IHSM 89 technology and discern differences in emulsification behaviour based on FFME formulation, 90 91 in terms of emulsion fat droplet size distribution, emulsion viscosity and accelerated physical stability, as a function of processing time. Moreover, the emulsification process was monitored 92 93 inline using a pressure drop approach, by applying the Hagen-Poiseuille equation. This approach allows for real-time monitoring of industrial emulsification processes, and provides 94 information as to when dosing of oils is complete, as well as the progression of the 95 emulsification process. 96

97 2. Materials and methods

98 2.1. Materials

99 Skim milk concentrate (SMC) and refined palm oil (RPO) were kindly provided by
100 Dairygold Food Ingredients (Mitchelstown, Ireland). Maltodextrin with a dextrose equivalent
101 (DE) value of 17 (MD17) was supplied by Corcoran Chemicals Ltd. (Dublin, Ireland). The
102 composition of the SMC is presented in Table 1. The water used throughout this study was
103 deionised water, unless stated otherwise.

104 *2.2. Emulsion formulation and preparation*

Emulsification was conducted at three protein concentrations, 7.7, 10.5 and 13% (w/w), 105 with a fixed fat content of $12.1 \pm 0.1\%$ (w/w), whereby the % (w/w) level is based on total 106 solids within a given system (*i.e.*, formulated emulsion or projected FFMP). Variations in 107 emulsion formulation to meet these protein concentrations were achieved through addition of 108 a fixed quantity of RPO and varying quantities of MD17 and water to SMC, as detailed in 109 Table 1, with a target solids content of $52.3 \pm 0.2\%$ (w/w) in all cases. These protein contents 110 were selected as the range in protein content for typical FFMP products is 14 to 24% (w/w) for 111 the low to high protein contents, respectively (Sharma et al., 2012). The predicted protein 112 content of powders produced from the prepared emulsions would be 14.2, 19.2 and 23.7% 113 114 (w/w) for the low-, medium- and high-protein systems, respectively, assuming that the final moisture content of the powder was 4% (w/w) in all cases (Table 1). 115

The configuration used for emulsification is shown in Fig. 1. The emulsification process was started by filling the closed-loop liquid system with the required amount of SMC to achieve the desired protein content for the investigated emulsion systems (Table 1), and initialising the progressive cavity pump (Torqueflow, Sydex, UK) at a volumetric flowrate of 675 L h⁻¹. Next, the inline high-shear mixer (IHSM), a YTRON-Z (1.50FC, YTRON Process Technology GmbH, Germany) operating at 100% capacity, yielding *ca*. 6,000 rpm, was initialised, and the custom-fabricated heat exchanger (Liam A. Barry Ltd., Cork, Ireland), in counter-current

configuration, was set to a temperature of 50°C. An overhead stirrer (RZR 2021, Heidolph 123 Instruments GmbH & Co. KG, Schwabach, Germany) at a speed of 1,000 rpm was used to 124 ensure rapid dispersion of MD17 powder and added water, and retained in place for the duration 125 of the emulsification process. The required mass of MD17 and water were carefully added to 126 the feed vessel over the top once the temperature of the recirculating SMC had reached 50°C 127 and the mixture was allowed to circulate through the system for a minimum of 30 min. 128 Subsequently, RPO was liquefied at a temperature of 50°C, the required mass was added to the 129 feed vessel over the top, and the mix was emulsified for up to 15 min (>50 passes through the 130 131 IHSM).

132 2.3. Emulsion droplet size characterisation

The changes in fat droplet size as a function of pass number (1, 3, 5, 10, 25 and 50 133 passes) through the IHSM were measured by static light-scattering using a Mastersizer 3000 134 (Hydro EV, Malvern Instruments, UK). Emulsion fat droplet size was reported as $d_{4,3}$ (*i.e.*, 135 volume-weighted mean droplet size), d_{10} (*i.e.*, cumulative 10% point of diameter), d_{50} (*i.e.*, 136 cumulative 50% point of diameter), d_{90} (*i.e.*, cumulative 90% point of diameter), droplet size 137 distribution data (DSD; volume vs. size class), and span (i.e., width of the droplet size 138 distribution). Eq. 1 was used in order to determine the times required to achieve the desired 139 number of passes of the emulsion through the IHSM (O'Sullivan et al., 2015): 140

141
$$t = \frac{V \times Pass \, number}{Q} \tag{1}$$

where *t* is the residence time (s), *V* is the volume within the system (m³), and *Q* is the volumetric flow rate (m³ s⁻¹).

144 2.4. Viscosity determination: comparison of calculated and experimental approaches

145 Viscosity was calculated from experimentally measured pressure drop (ΔP) readings, and compared to experimentally measured viscosity, in order to validate the calculated 146 viscosity results, using a similar approach to that described by O'Sullivan et al. (2017). 147 Pressure drop was recorded for the emulsification process, at all protein: fat ratios, and was 148 recorded using a pair of pressure transducers (PR-33X, Keller, UK), positioned 1.08 m apart 149 (Fig. 1). Pressure differential data was collected before dosage of molten RPO and for up to 15 150 min during the emulsification process. Calculated viscosity values were determined from Eq. 151 2, the Hagen-Poiseuille equation, using experimentally-measured pressure drop values, as 152 follows (Douglas et al., 2005; O'Sullivan et al., 2017): 153

154
$$\eta_{calculated} = \frac{\pi \Delta P d^4}{128LQ}$$
 (2)

where $\eta_{calculated}$ is the calculated viscosity (Pa.s), ΔP is the pressure differential across a given straight section of pipeline (Pa), *d* is the internal diameter (19.05 mm), *L* is the length over which the pressure drop was recorded (1.08 m), and *Q* is the volumetric flow rate (m³ s⁻¹).

The experimental viscosity ($\eta_{experimental}$) was measured for all emulsion systems, after 1 158 and 50 passes, using a rotational viscometer (RST-CC Touch[™], Brookfield AMETEK, 159 Middleboro, MA, USA) equipped with a cup-and-bob geometry. Apparent viscosity was 160 measured at 50°C (*i.e.*, the mean temperature at which emulsification was conducted; Section 161 2.2). A shear rate of 300 s⁻¹ was used for viscosity determination, as this was determined to be 162 similar to the shear rate in the pipeline between the pair of pressure transducers; the calculated 163 shear rate within the 1.08 m section from which the pressure drop was recorded was 275 s⁻¹, 164 determined using Eq. 3 (Douglas et al., 2005): 165

166
$$\dot{\gamma} = \frac{8v}{d}$$
, where $v = \frac{Q}{A}$ (3)

167	where $\dot{\gamma}$ is the shear rate (s ⁻¹), <i>d</i> is the internal diameter (19.05 mm), <i>v</i> is the average velocity
168	(m s ⁻¹), Q is the volumetric flowrate (m ³ s ⁻¹), and A is the cross-sectional area (m ²).

169 2.5. Accelerated physical stability analysis of emulsions

Separation rates of FFMEs collected after 1 and 50 passes of aqueous and oil phases 170 through the IHSM were measured using an analytical centrifuge (LUMiSizer, L.U.M. GmbH, 171 Berlin, Germany). The principle of analysis by LUMiSizer has been detailed by Lerche and 172 Sobisch (2011). Stability of emulsions to separation (i.e., creaming and sedimentation) driven 173 by difference in the density between fat globules, undissolved powder and protein aggregates 174 and the aqueous phase was determined at 23°C and 563 g over 500 min (i.e., 8 h 20 min) as 175 detailed by Shimoni et al. (2013). Separation rates were calculated from integral transmission 176 (IT) profiles using the initial linear ($R^2 \ge 0.95$) region of the slope of the plot of integral 177 transmission vs. measurement time. Separation profiles (i.e., the Space- and Time-resolved 178 Extinction Profiles, STEP; Lerche and Sobisch, 2011) were collected at 10 min intervals during 179 accelerated testing of emulsions to give information on changes in the light transmission 180 through the measurement cell as a function of the specific position in the cell and, effectively, 181 indicating progressive migration of emulsion components (i.e., creaming and/or 182 sedimentation). 183

184 *2.6. Statistical analysis*

Presented data are the average and standard deviation of at least three repeat measurements, and from a single production run of SMC, RPO and MD17. Student's t-test with a 95% confidence interval analysis was performed using Microsoft Excel and was used to assess the significance of the results obtained, whereby t-test differences with P < 0.05 were considered statistically significant.

190 **3. Results and discussion**

3.1. Effect of pass number through the inline high-shear mixer (IHSM) on fat droplet size distribution

The effect of pass number through the IHSM (*i.e.*, residence time within the shear field) 193 on fat droplet size distribution was assessed for low-, medium- and high-protein FFMEs (Fig. 194 2 and Table 2). After a single pass through the IHSM, large fat droplets were found; the 195 medium-protein FFME yielded the smallest initial droplets ($d_{4,3}$ of 6.67 ± 0.31 µm), while the 196 high-protein FFME yielded the largest initial droplet size ($d_{4,3}$ of 9.62 ± 0.79 µm). This may 197 be explained by the fact that moderate concentrations of protein allow for more efficient 198 adsorption and stabilisation of oil-water interfacial layers, yielding smaller emulsion droplets 199 200 (Beverung et al., 1999; O'Sullivan, Beevers et al., 2015). As these samples were further processed (*i.e.*, with increasing pass number), the size of the fat droplets (in particular d_{50} and 201 d_{90} ; Table 2) decreased significantly (P < 0.05), for all protein contents investigated. 202

Furthermore, the extent of droplet size reduction was greatest for the low-protein 203 emulsions, in terms of d_{50} and d_{90} , throughout the entire process. This behaviour was attributed 204 to the higher viscosity of the continuous phase of those systems in comparison to that of the 205 medium- and high-protein samples, allowing for greater ease of disruption of fat droplets (Lee 206 et al., 2013; Walstra, 1993). A higher viscosity difference between the continuous and 207 dispersed phases (*i.e.*, viscosity ratio), results in enhanced droplet breakup within the turbulent 208 flow regimes observed for the IHSM (Walstra & Smulders, 2000). Furthermore, the primary 209 mode of droplet breakup within the IHSM results from the high degree of turbulence, which 210 causes chaotic velocity fields, resulting in turbulent eddies, characterised by the Kolmogorov 211 length scale (Walstra, 1993). 212

In addition, in all cases, and for any given time point in the process, a bimodal size 213 distribution was observed (Fig. 2), in which the micron-sized peak (ca. 5 µm after 50 passes) 214 was ascribed to emulsion fat droplets, whereas the submicron peak (ca. 250 nm) was associated 215 with casein micelles, the dominant protein fraction of SMC (O'Sullivan et al., 2017). After 25 216 passes through the IHSM, the low-protein (7.7% w/w) emulsion had droplets $\leq 10 \ \mu m$ (Fig. 217 2a), while droplets $> 10 \,\mu\text{m}$ were still present in the medium- (Fig. 2b) and high-protein (Fig. 218 219 2c) emulsions even after 50 passes through the IHSM. It is also worth noting that the lowprotein emulsion had the narrowest droplet size distribution (DSD; Fig. 2a), irrespective of the 220 221 number of passes through the IHSM, as also evident from the lowest span values for this emulsion (Table 2), compared to medium- and high-protein emulsions. This is thought to be 222 associated with the higher viscosity of the continuous phase of the low-protein content FFME, 223 in comparison to the other protein contents. The reduction of emulsion droplet size as a function 224 of pass number was similarly demonstrated for a range of other emulsification processes, 225 including IHSMs (Hall et al., 2011), continuous ultrasonic processors (O'Sullivan et al., 2015), 226 high-pressure valve homogenisers (Lee & Norton, 2013) and microfluidizers (Lee & Norton, 227 2013). 228

3.2. Inline assessment of emulsification using the pressure drop approach

The calculated viscosity ($\eta_{calculated}$) as a function of pass number (up to 50 passes) was 230 231 investigated and is shown in Fig. 3 for FFMEs prepared at low-, medium- and high-protein concentrations. Upon addition of molten RPO to the emulsification system (Fig. 1), there was 232 a significant increase (P < 0.05) in $\eta_{calculated}$ for all of the investigated formulations, where this 233 behaviour was ascribed to the increased solids content within the system, resulting in an 234 increased pressure differential and thus $\eta_{calculated}$ (Douglas *et al.*, 2005; O'Sullivan *et al.*, 2017). 235 Following the addition of fat, $\eta_{calculated}$ decreased marginally as a function of pass number, in 236 particular for the medium-protein FFME. This behaviour was attributed to the reduction of fat 237

droplet size, which is known to result in a reduced viscosity for emulsion systems (McClements, 2005). Thus, the emulsification process exhibited two distinct stages in all instances, an initial significant (P < 0.05) increase, followed by a gradual reduction to a final viscosity value. These distinct stages correspond to: (i) an increase in the solids content of the system due to the introduction of molten fat to the skim milk concentrate (SMC), and (ii) size reduction of fat droplets with successive passes through the IHSM.

244 Furthermore, when comparing $\eta_{calculated}$ values after 50 passes for each FFME formulation, the low-protein emulsion exhibited, unexpectedly, the highest viscosity value 245 $(36.5 \pm 1.3 \text{ mPa.s})$, followed by the high-protein emulsion, with a marginally lower viscosity 246 value $(34.7 \pm 2.2 \text{ mPa.s})$, and the medium-protein sample, which had a significantly lower (P 247 < 0.05) viscosity (29.2 \pm 0.7 mPa.s), in comparison to both the low- and high-protein systems. 248 Even though all of the systems had the same solids content (52.5% w/w; Table 1), the factor 249 which dictated the resultant value of $\eta_{calculated}$ was thought to be the concentration of MD17, 250 rather than the protein content. MD17 has an average molecular weight of 24.9 kDa (Chen & 251 O'Mahony, 2016; Rong et al., 2009), and maltodextrin has a highly branched structure 252 consisting of D-glucose monomer units (Avaltroni et al., 2004; Chronakis, 1998; Wang & 253 Wang, 2000); in addition, individual molecules of MD17 interact with one another, 254 contributing to increases in viscosity with increasing concentration (Avaltroni et al., 2004; 255 Morris *et al.*, 1981). The intrinsic viscosity ($[\eta]$; *i.e.*, hydrodynamic volume) of MD17 is 256 significantly greater than that of the proteins in SMC, ca. 80:20 mixture of casein micelles and 257 whey protein, the same as observed in milk protein isolates (MPI) (O'Connell & Flynn, 2007; 258 Vos *et al.*, 2016), whereby the $[\eta]_{MD17}$ was 3.5 dL g⁻¹, in comparison to $[\eta]_{MPI}$ which had a 259 value of 0.59 dL g⁻¹ (Avaltroni *et al.*, 2004; O'Sullivan *et al.*, 2014). The significantly (P < 260 0.05) higher value of $[\eta]_{MD17}$ highlights that MD17 would have a more pronounced effect on 261 the resultant viscosity of FFMEs than the protein component. Thus, the higher concentration 262

of MD17 in the low-protein content emulsion yielded the highest viscosity and, as the concentration of MD17 decreased, and that of protein increased, there was a significant (P <0.05) decrease in viscosity. Moreover, as the concentration of MD17 further decreased, and the concentration of protein increased, the protein component becomes the dominant influencer of viscosity, in comparison to MD17; nevertheless, the resultant viscosity remained lower than that of the low-protein emulsion system (Fig. 3).

The validity of the $\eta_{calculated}$ results was assessed through direct comparison of 269 experimentally obtained viscosity values measured at a shear rate of 300 s⁻¹, a value close to 270 that at which the pressure drop was measured (275 s⁻¹), and at the average temperature recorded 271 during emulsification (50°C). The values of $\eta_{calculated}$ and experimental viscosity ($\eta_{experimental}$) 272 for all of the investigated FFME systems, after 1 and 50 passes, are shown in Table 3. The 273 trend of $\eta_{experimental}$ for all of the FFMEs is comparable to that of $\eta_{calculated}$, whereby the low-274 protein system possessed the highest apparent viscosity and the medium-protein emulsion 275 exhibited the lowest viscosity, for the same reasons as previously discussed, associated with 276 differences in MD17 concentration. Furthermore, the viscosity values for 1 pass were 277 significantly (P < 0.05) lower than those at 50 passes, which is in agreement with $\eta_{calculated}$ 278 values as a function of time (Fig. 3). This behaviour is ascribed to either the fact that the RPO 279 has not had sufficient time to form a uniform emulsion after a single pass (< 14 s), or potential 280 increased levels of hydration of MD17 resulting from the shearing process. 281

A comparison of the $\eta_{calculated}$ and $\eta_{experimental}$ values for all FFME systems highlight that there is a discrepancy in the values, by a factor of *ca*. 1.25, whereby the calculated value represents an overestimation in all instances. This observed difference between calculated and experimental values was ascribed to the nature of the Hagen-Poiseuille equation, which assumes that the fluid exhibits Newtonian behaviour, whereas it has been established that highly concentrated (52.5% solids, w/w) emulsion systems demonstrate pseudoplastic

rheological behaviour (O'Sullivan *et al.*, 2016; Pal, 1996, 2011). A similar trend was observed
by O'Sullivan *et al.* (2017) for the induction and dissolution of dairy powders, whereby the
difference between calculated and experimental viscosity values was a factor of 2, which was
also ascribed to the non-Newtonian behaviour of dairy solutions.

292 *3.3. Accelerated physical stability of emulsions*

Differences in the extent of phase separation in FFME systems after 1 and 50 passes 293 through the IHSM tested under accelerated conditions (563 g for 500 min) were clear from the 294 space- and time-resolved extinction profiles (STEP; Fig. 4). After the 1st pass through the 295 IHSM, only limited differences in phase separation were observed between all emulsions. More 296 pronounced differences in separation were observed for emulsions after 50 passes through the 297 IHSM, where the low-protein (7.7%, w/w) emulsion displayed lowest separation, followed by 298 emulsions with high- (13%, w/w) and medium-protein (10.5%, w/w) levels (Fig. 4). Separation 299 of formulations was identified as being mostly due to the migration of fat globules towards the 300 top of the measuring cell (*i.e.*, creaming) as evidenced by a progressive appearance of a cream 301 layer and only a limited sediment build-up in all samples (Fig. 4). Creaming and sedimentation 302 were reduced on progressive recirculation through the IHSM system, due to decreases in the 303 size of fat globules and enhanced hydration of the MD17 powder (O'Sullivan et al., 2016). 304

Similar emulsion separation trends were observed for the integral transmission (IT) profiles (Fig. 5); the IT represents separation in the samples due to both creaming (upward movement of the less dense phase, i.e., fat droplets, and downward movement of the more dense solutes, i.e., maltodextrin and protein). The evolution of separation increased in the order of low-protein 50th pass < high-protein 50th pass < medium-protein 50th pass < high-protein 1st pass < medium-protein 1st pass < low-protein 1st pass. Despite lack of significant differences in the initial (*i.e.*, first 45 min) slopes of increasing transmission for emulsions after 50 passes

through the IHSM (Table 2), the overall (*i.e.*, during 500 min) separation of the low-protein emulsion was lower than that of both medium- and high-protein emulsions after 50 passes (Fig. 5). This can be explained by the greater population of smaller particles (*i.e.*, fat globules; Fig. 2, Table 2) in the low-protein content emulsion after 50 passes, compared to the other emulsions after 50 passes, causing divergence of the IT profiles after initial movement of the larger particles (*i.e.*, bigger particles move first and smaller particles move more slowly).

The results for the accelerated emulsion separation closely correlate with those obtained for DSD and apparent viscosity of the FFME systems, whereas, in accordance with Stoke's Law, emulsions with largest droplet size and lowest viscosity also displayed the most rapid separation. The low-protein emulsion after 50 passes had the highest apparent viscosity, compared to the other emulsions (Table 3), further enhancing stability of the emulsion to density-driven separation.

324 4. Conclusions

Inline high-shear mixing (IHSM) was shown to be an effective approach for the 325 326 preparation of fat-filled milk emulsions (FFMEs). The most effective emulsification, as a function of pass number, was achieved for the low-protein FFME, as observed by the formation 327 of smaller emulsion droplets, which was ascribed to the enhanced droplet breakup due to the 328 increased viscosity differential between the dispersed and continuous phases. Inline 329 measurement of pressure drop is thus an effective approach for monitoring real-time 330 emulsification kinetics of refined palm oil (RPO) in skim milk concentrate (SMC). Pressure 331 drop data was used to determine real-time viscosity, by means of the Hagen-Poiseuille 332 equation; after emulsification, the low-protein FFME exhibited the highest viscosity in 333 comparison to the other systems, which was ascribed to lower and narrower DSD and to the 334 higher content of MD17 and its associated higher intrinsic viscosity in comparison to the 335

protein component of the formulations. The lowest viscosity was exhibited by the mediumprotein FFME, associated with the reduction in MD17 concentration. The emulsification process exhibited two distinct phases as observed by pressure drop results: (i) initial injection of fat, and (ii) fat droplet reduction in the shear mixing field.

340

341 Acknowledgements

The authors would like to acknowledge the Dairy Processing Technology Centre 342 (DPTC), an Enterprise Ireland initiative, for financial support and permission to publish this 343 work. This work was supported by the Irish State through funding from the Technology Centres 344 programme (Grant Number TC/2014/0016). The authors would like to thank Dr Olga 345 Mihailova of Unilever Research (Port Sunlight, UK) for assistance with respect to data 346 processing. The authors would also like to thank Mike Barry and Kevin McEvoy of Liam A. 347 Barry Ltd. (Cork, Ireland) for the custom fabrication of many of the stainless steel components 348 of the experimental setup. 349

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466

1 Fig. 1.



1 Fig. 2.



2

1 Fig. 3.



1 Fig. 4.





1 Figure captions

Fig. 1. Schematic representation of the experimental configuration employed, showing the
inline high-shear mixer, heat exchanger, pressure transducers, batch vessel and pump.

Fig. 2. Changes in oil droplet size distribution as a function of pass number through the inline
high-shear mixer, showing data for 1 (solid line), 5 (long-dashed line), 25 (medium-dashed
line), and 50 (short-dashed line) passes after dosing of refined palm oil for: (a) low-protein fatfilled milk emulsions (FFME), (b) medium-protein FFME, and (c) high-protein FFME. The
concentration of refined palm oil in all cases was 12% (w/w).

9 Fig. 3. Calculated viscosity upon addition of molten refined palm oil to the system as a function
10 of time for low-protein fat-filled milk emulsions (FFME) (solid line), medium-protein FFME
11 (long-dashed line), and high-protein FFME (short-dashed line). The concentration of refined
12 palm oil in all cases was 12% (w/w).

Fig. 4. Emulsion separation profiles for low- (L), medium- (M) and high-protein (H) fat-filled 13 milk emulsions (FFMEs) after 1 and 50 passes through the inline high-shear mixer. The profiles 14 demonstrate changes in the transmission of light trough the sample cell due to migration of its 15 16 component under centrifugal acceleration. The sample is contained between the position 110 mm (top of the cell) and position 129 mm (bottom of the cell). The evolution of the transmission 17 profiles over the duration of the analysis is represented by the arrow (b), where the phase 18 boundary progressively moves towards the bottom of the cell while the thickness of the cream 19 layer increases (a) and the sediment layer builds-up (c). Colours indicate the sequence of the 20 profiles (RED profiles were collected early, first profile at time 0 min; GREEN profiles were 21 22 collected late in separation, last profile collected at time 500 min – please refer to on-line version for full colour Figure). 23

Fig. 5. Separation profiles expressed as integral transmission as a function of time for fat-filled
milk emulsions (FFMEs) with low- (circle), medium- (triangle) and high-protein (square) after
1 (solid fill) and 50 (no fill) passes through the inline high-shear mixer as measured using the
LUMiSizer analytical centrifuge.

1 Table 1.

- 2 Composition of skim milk concentrate (SMC), low-, medium- and high-protein fat-filled milk
- 3 emulsions (FFME), and calculated composition of resultant low-, medium- and high-protein
- 4 content fat-filled milk powders (FFMP).

		Fat-F	illed Milk Emu	Fat-Filled Milk Powders				
	SMC	Low	Medium	High	Low	Medium	High	
Protein (%)	16.2	7.7	10.5	13	14.2	19.2	23.7	
Fat (%)	0.4	12.2	12.2	12	22.4	22.3	22.1	
Lactose (%)	23.1	11.4	14.9	18.6	21	27.2	34	
Maltodextrin (%)	0	20.3	14.2	8	37.4	26	14.6	
Lactose + Maltodextrin (%)	23.1	31.7	29.1	26.6	58.4	53.2	48.6	
Ash (%)	1.1	0.5	0.7	0.9	1	1.3	1.6	
Water (%)	59.2	47.9	47.5	47.5	4	4	4	

5

- 6 **Table 2.**
- 7 Effect of pass number (1, 5, 25 and 50) through the inline high-shear mixer on $d_{3,2}$ (*i.e.*, Sauter diameter), $d_{4,3}$ (*i.e.*, volume-weighted mean
- 8 diameter), d_{10} , d_{50} , d_{90} , span, and separation rates, calculated from the initial linear response ($R^2 \ge 0.95$) of the slope of plots of integral transmission
- 9 vs. measurement time, for low-, medium- and high-protein fat-filled milk emulsions (FFMEs).

C

Protein Content (% w/w)	Pass (-)	d _{4,3} (μm)	d ₁₀ (μm)	d ₅₀ (μm)	d ₉₀ (μm)	Span (-)	Rate of initial transmission increase (%/h)
7.7	1	9.62 ± 0.79	0.45 ± 0.01	9.65 ± 0.05	17.4 ± 0.13	1.91 ± 0.02	7.69 ± 0.43
	5	5.47 ± 0.32	0.51 ± 0.01	4.91 ± 0.03	10.5 ± 0.04	2.04 ± 0.01	-
	25	3.08 ± 0.09	0.49 ± 0.02	2.65 ± 0.04	6.35 ± 0.05	2.17 ± 0.03	-
	50	2.31 ± 0.03	0.49 ± 0.03	2.11 ± 0.02	4.41 ± 0.03	1.82 ± 0.02	5.60 ± 0.05
10.5	1	6.67 ± 0.31	0.43 ± 0.04	5.76 ± 0.07	16.8 ± 0.09	2.89 ± 0.05	6.85 ± 0.35
	5	4.89 ± 0.08	0.51 ± 0.05	3.73 ± 0.04	12.3 ± 0.02	3.16 ± 0.02	-
	25	4.05 ± 0.02	0.52 ± 0.03	2.83 ± 0.02	8.21 ± 0.05	2.73 ± 0.01	-
	50	3.99 ± 0.06	0.65 ± 0.03	2.82 ± 0.01	6.85 ± 0.06	2.26 ± 0.02	5.47 ± 0.03
13	1	9.41 ± 0.87	0.37 ± 0.01	6.58 ± 0.09	15.4 ± 0.13	2.34 ± 0.06	6.16 ± 0.07
	5	6.22 ± 0.51	0.39 ± 0.01	4.15 ± 0.04	10.8 ± 0.05	2.42 ± 0.08	-
	25	4.52 ± 0.31	0.41 ± 0.01	3.04 ± 0.02	8.11 ± 0.07	2.57 ± 0.05	-
	50	3.74 ± 0.19	0.36 ± 0.01	2.52 ± 0.04	7.09 ± 0.06	2.74 ± 0.11	5.35 ± 0.12

11 **Table 3.**

12 Comparison of calculated viscosity (1 and 50 passes after dosing of molten refined palm oil) 13 and experimentally measured viscosity (at a shear rate of 300 s^{-1}) for fat-filled milk emulsions 14 with low-, medium- and high-protein contents. The concentration of refined palm oil in all 15 cases was 12% (w/w).

Number of	Protein Content	$\eta_{calculated}$	$\eta_{experimental}$
Passes			
(-)	(% w/w)	(mPa	a.s)
	7.7	29.1 ± 1.1	22.6 ± 0.2
1	10.5	21.8 ± 0.8	13.5 ± 0.5
	13	23.9 ± 1.7	26.5 ± 2.9
	7.7	36.5 ± 1.3	33.4 ± 0.5
50	10.5	29.2 ± 0.7	15.9 ± 1.5
	13	34.7 ± 2.2	27.6 ± 3.2

16