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ORIGINAL
RESEARCH

Influence of protein content and profile on the processing characteristics and physical properties of model infant formula powders

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Formulas were designed and produced with protein contents of 10, 14 and 18 g/100 g and whey protein:casein ratios of 60:40, 40:60 and 20:80. Protein content and whey protein:casein ratio did not significantly affect the volume mean diameter (D[4,3]) of milk fat globules during processing; however, increasing protein content and decreasing whey protein:casein ratio resulted in increased viscosity during processing. The free fat content of the powders decreased with increasing protein content. Particle and bulk densities of powders with whey protein:casein ratio of 20:80 were higher than that of powders with whey protein:casein ratio of 60:40.

Keywords Infant formula, Whey, Casein, Protein, Viscosity, Homogenisation, Powder.

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INTRODUCTION

Infant formula is a general term used to collectively describe a broad range of nutritional products designed to be consumed during infancy and childhood (Montagne *et al*. 2008). Classifications stemming from regulations and guidelines governing infant Formulas allow for clear definitions of the various categories of these nutritional products. First age Formulas are designed to be consumed from birth to 6 months, follow-on Formulas from 6 to 12 months, and growing-up milk products are intended for use from 12 months onwards (O'Callaghan *et al*. 2011).

Infant Formulas incorporate complex mixtures of proteins, lipids, carbohydrates, vitamins and minerals in addition to other minor ingredients, to best match the composition of human milk. Despite significant differences between human milk and bovine milk, mainly with respect to the protein, carbohydrate and ash constituents, bovine milk fractions and derivatives are commonly used in the manufacture of infant formula (Nasirpour *et al*. 2006). Skim milk powder (SMP), whole milk powder (WMP) and/or milk protein concentrate (MPC) typically contribute the casein component of infant Formulas, while demineralised

whey powder (DWP), whey protein concentrate (WPC) and/or whey protein isolate (WPI) ingredients typically contribute whey proteins (Montagne *et al*. 2008; McCarthy *et al*. 2012; McSweeney *et al*. 2013; Buggy *et al*. 2016). These ingredients contribute to the protein content and profile of infant Formulas, which may be adjusted dependent on product category. The ingredients may be used individually or in combination to achieve target whey protein:casein ratio of infant Formulas, in most cases with the aim of matching the whey protein:casein ratio of human milk, which ranges from 80:20 (early lactation) to 60:40 (late lactation) (Lönnerdal *et al*. 2017).

Infant formula, in powder format, can be manufactured by wet mixing or dry blending of ingredients, or a combination of both technological approaches (McSweeney 2008). In wet mixing, the ingredients are mixed and hydrated, followed by heat treatment, homogenisation, evaporation and spray drying. Following mixing and hydration of the ingredients, heat treatment is applied to the liquid mix to ensure microbiological safety of the final product, and may be achieved using indirect (e.g. plate/tubular heat exchanger) or direct (e.g. steam injection/infusion) heat treatment (Montagne *et al*. 2008; McSweeney *et al*. 2013; Murphy *et al*. 2013).

During heat treatment, physical changes to the liquid mix, such as increased viscosity, may occur. Liquid mixes with higher protein contents have been shown to have higher viscosity than liquid mixes with higher lactose contents (Westergaard 2004; Schuck *et al*. 2005; McCarthy *et al*. 2013). Whey proteins are particularly susceptible to denaturation at high temperatures due to their compact globular structure. This denaturation can lead to their structure unfolding, resulting in association of whey proteins with themselves and other milk proteins, which may ultimately lead to increased viscosity (Simmons *et al*. 2007). Lactose has been shown to have an inhibitory effect on protein denaturation by increasing the temperature at which whey protein denaturation occurs (Murphy *et al*. 2014). In comparison, caseins are much more heat stable and are capable of withstanding heat treatments exceeding those used in infant formula manufacture (e.g. 85 °C × 22 s) with minimal effects on their physical structure (Murphy *et al*. 2014); however, they are known to form more viscous solutions in comparison with whey proteins at similar concentration and at temperatures lower than the denaturation temperatures (O'Regan *et al*. 2009).

During homogenisation, proteins migrate and adsorb at the lipid–water interface, resulting in stabilisation and protection of lipid droplets from coalescence. Numerous studies have investigated the effect of protein content on emulsion formation during homogenisation of the liquid mix (Floury *et al*. 2000; McCarthy *et al*. 2012). These studies reported that as protein content decreased (i.e. protein:fat ratio decreased), the amount of protein available for emulsification also decreased, resulting in larger fat droplets, droplet coalescence and in some cases increased free fat content in the finished powder. Previous studies into the emulsifying capability of different proteins identified casein proteins as having preferential adsorption to the fat droplets over whey proteins during homogenisation, therefore conferring better emulsifying capabilities of the former (Britten and Giroux 1991; Sourdet *et al*. 2002; Liang *et al*. 2016).

In recent years, the increased volume of scientific knowledge available on breast milk composition has resulted in increasingly complex infant formulas and considerable growth in different product categories. Research has continued into the effects of fundamental compositional changes on processing characteristics, and this study aimed to supplement currently available knowledge, which would support the design, formulation and manufacture of infant formulas with modified protein contents and profiles to better match the composition of human milk and nutritional needs of the infants. The objective of the present study was to determine the effects of increasing protein content, in combination with altering protein profile (whey protein:casein ratio), of a model infant formula on its processing characteristics, specifically viscosity and fat globule size, in addition to physical properties of the resultant powder.

MATERIALS AND METHODS

Materials
Whey protein concentrate (WPC), based on sweet whey, was sourced from Arla Food Ingredients (Viby, Denmark) with a protein content of 34.6 g/100 g as determined by Kjeldahl analysis (IDF 2001). Lactose was sourced from Arla Food Ingredients (Viby, Denmark). Skimmed milk powder (SMP) was sourced from Dairygold Food Ingredients (Co. Cork, Ireland) with a protein content of 36.6 g/100 g as determined by Kjeldahl analysis (IDF 2001). A lipid blend was prepared using a combination of palm, soya bean and sunflower oil, along with soya bean lecithin, in the proportions of 46, 49, 4 and 1%, respectively. The lipid blend was selected and designed based on a detailed review of typical blends used in commercial infant Formulas, follow-on Formulas and growing-up milks. Potassium hydroxide and citric acid were used, when necessary, to adjust pH to target 6.8 at end of batch preparation.

Experimental design
An experimental design was constructed using MiniTab 17 Design of Experiment (DOE) function. A response surface model with two factors (protein concentration and whey protein:casein ratio) and three levels was used; see Table 1 for DOE. The four corner points, and the centre point formulations, were produced in duplicate, and all trials were conducted in a randomised order. Three different protein concentrations (10, 14 and 18 g/100 g) and three different whey protein-to-casein ratios (60:40, 40:60 and 20:80) were used in the study (Table 1). To identify the most relevant protein contents for further analysis, preliminary work involved the measurement of the protein content of a range (15 samples analysed) of commercial infant Formulas (0–6 months), follow-on Formulas (6–12 months) and growing-up milks (>12 months). The measured protein contents of the infant

Table 1 Design of experiments for model formulations with different protein contents and whey protein-to-casein ratios.

Formula	Protein content (g/100 g)	Whey protein:casein ratio	Number of replicates
10A	10	60:40	2
10B	10	40:60	1
10C	10	20:80	2
14A	14	60:40	1
14B	14	40:60	2
14C	14	20:80	1
18A	18	60:40	2
18B	18	40:60	1
18C	18	20:80	2

formula products were ~10 g/100 g, while the follow-on formulas and growing-up milks contained 14-18 g/100 g protein. SMP and WPC35 were used in combination to achieve the target whey protein:casein ratios.

Production of model formulas

Forty-litre batches of each formula were prepared as follows: one third of the lipid blend (tempered at 45 °C) was first added to distilled hot water (~75 °C) to reduce foaming upon addition of the macronutrient ingredients. The SMP, WPC and lactose ingredients were then added individually, in that order, at 5 min intervals, and mixed using a Silverson L4RT Mixer (Silverson Machines Ltd., Chesham, UK), followed by the remaining two thirds of the lipid blend. After 10 min of mixing, the pH of the mix was measured at 50 °C and adjusted (if required) to target pH 6.8, using either potassium hydroxide to increase the pH or citric acid to decrease the pH, after which the mix was left mixing for 10 min prior to further processing.

The mix was thermally processed at 100 °C × 30 s using a Microthermics (Model 25HV; North Carolina, USA) tubular heat exchanger and homogenised using an in-line two-stage valve homogeniser (Model NS20006H; GEA Niro Soavi, Parma, Italy) using first- and second-stage pressures of 13.8 and 3.45 MPa. The mix was held in storage overnight at 4–8 °C, with mixing using an overhead stirrer (Euro-ST Digital; IKA®-Werke GmbH & Co. KG, Janke & Kunkel-Str. 10, 79219 Staufen, Germany) equipped with a propeller (3-blade, R 1381 Propeller; IKA®-Werke GmbH & Co. KG, Janke & Kunkel-Str. 10, 79219 Staufen, Germany) set to 30 revolutions per minute (rpm). Following overnight storage, the mix was pre-heated to 65 °C prior to spray drying using a pilot-scale Anhydro spray dryer (Model Plant No. 3 Type IKA; Copenhagen, Denmark) with a typical water evaporation rate of 20 L/h. The dryer inlet and outlet temperatures were held constant at 185 °C and 90 °C, respectively.

In-process analysis

The mix was monitored for pH, temperature, total solids (TS), viscosity and fat globule size at the post-mixing (PM), post-processing (PP), post-overnight (PO) and pre-drying (PD) stages during liquid formulation processing.

pH, temperature and total solids

The pH was measured using a 330i pH meter (WTW Germany). Prior to use, the meter was calibrated using pH 4.01, 7.00 and 10.01 technical pH buffers (WTW Germany). The temperature was measured using a PT100 thermometer (Sensortech Ltd., Ireland). TS was measured using a Turbo Smart 5 Microwave Moisture/Solids Analyser (CEM Corporation, Matthews, North Carolina, USA).

Viscosity

Viscosity was measured using an AR-G2 controlled stress rheometer (TA Instruments, Hertfordshire, UK) equipped with concentric cylinder geometry. The samples were pre-sheared at 300 s^{-1} for 1 min, followed by equilibration for 2 min. A shear rate sweep was then applied from 1 to 300 s^{-1} over 3 min, followed by holding at 300 s^{-1} for 1 min. The average apparent viscosity measured at 300 s^{-1} was reported.

Mean fat globule size

The mean fat globule size (FGS; [D4,3]), also known as the volume mean diameter, was measured using laser light scattering with a Mastersizer S laser diffraction instrument (Malvern Instruments Ltd., Grovewood Rd, Malvern WR14 1XZ, UK) equipped with a 300 RF lens. Distilled water was used as the dispersing medium. The optical parameters chosen were particle and dispersant refractive indices of 1.46 and 1.33, respectively.

Chemical composition of powders

Total protein was analysed using the Dumas method, with a nitrogen-to-protein conversion factor of 6.25 (AOAC International 2005b). Total fat was analysed by the base hydrolysis method (AOAC International 2005c). Total ash was measured by combustion (AOAC International 2005a). Total lactose content was calculated by difference in weight as described by Kelly *et al.* (2016). The free fat content of the powders was determined using the GEA Niro Analytical Method (GEA 2005a). Water content and water activity (a$_w$) of the powders were determined as described by Kelly *et al.* (2016). Free water content of the powders was determined using a halogen rapid moisture analyser (HR-83 Halogen; Mettler Toledo, Switzerland). Water activity (a$_w$) was measured with a Novasina LabMaster a$_w$ water activity meter (Novatron Scientific Ltd., West Sussex, UK).

Bulk density and particle density of model infant formula powders

Bulk density of powders was determined according to the GEA Niro Analytical Method (GEA 2006b). Powder particle density was measured according to the GEA Niro Analytical Method (GEA 2006a) using a pycnometer (AcuPyc 1340; Micromeritics, Norcross, Georgia, USA).

Statistical data analysis

Analysis of variance (ANOVA) was carried out using the general linear model (GLM) in Minitab 17 (Minitab Ltd., Coventry, UK). Effects were deemed statistically significant if $P < 0.05$. All analyses were performed in at least duplicate.

RESULTS AND DISCUSSION

Chemical composition of powders

The chemical composition of the powders is provided in Table 2. As per the study design, the protein content of the formulas with different protein targets was found to be significantly different ($P < 0.05$), while there were no

Table 2 Protein, fat, lactose, ash and free fat content of model infant formula powders with target protein contents of 10, 14 and 18 g /100 g and whey protein:casein ratios of 60:40 (A), 40:60 (B) and 20:80 (C).

	Protein	Fat	Lactose	Ash	Free fat
	(g/100 g)				
10A	10.1 ± 0.00[d]	19.8 ± 0.20[a]	66.3 ± 0.16[a]	2.36 ± 0.01[d]	1.08 ± 0.08[ab]
10B	10.1[d]	19.9[a]	66.9[a]	2.42[d]	1.25 ± 0.05[a]
10C	10.1 ± 0.00[d]	19.8 ± 0.20 [a]	66.2 ± 0.69[a]	2.42 ± 0.01[d]	1.00 ± 0.07[abc]
14A	13.8[c]	20.2[a]	61.1[ab]	3.14[c]	1.05 ± 0.05[abc]
14B	14.0 ± 0.04[bc]	20.2 ± 0.10 [a]	61.3 ± 0.20[ab]	3.18 ± 0.01[c]	0.88 ± 0.13[bc]
14C	14.1[b]	19.0[a]	62.1[ab]	3.29[c]	1.00 ± 0.00[abc]
18A	17.8 ± 0.04[a]	20.1 ± 0.05 [a]	56.4 ± 0.12[b]	3.99 ± 0.02[b]	0.80 ± 0.07[c]
18B	17.8[a]	20.3[a]	56.2[b]	4.10[ab]	0.95 ± 0.05[abc]
18C	18.0 ± 0.03[a]	19.2 ± 1.50 [a]	56.9 ± 1.56[b]	4.20 ± 0.02[a]	0.78 ± 0.13[c]

Duplicate trials of 10A, 10C, 14B, 18A and 18C, and individual trials of 10B, 14A, 14C and 18B. Values are means ± standard deviation; values within a column not sharing a common superscript differ significantly (*P* < 0.05).

significant differences (*P* > 0.05) in the fat content of the powders. Lactose was used as a flexing agent to maintain solid content of the formulas with different protein contents, and hence, the lactose content of powders targeting 10 and 18 g protein per 100 g was found to be significantly different (*P* < 0.05). Powders with higher protein content had higher addition levels of WPC and/or SMP and both ingredients have higher ash contents than lactose, resulting in higher ash content in the finished powder. The free fat content of the powders ranged from 0.78 to 1.25 g/100 g, with a reduction in free fat apparent with increasing protein content. This trend was explained further by comparing the protein:fat ratio of the powders, that is the trend of increasing protein:fat ratio (ranging from 0.51 to 0.94) associated with decreasing free fat content. A previous study by McCarthy *et al*. (2013) reported that the free fat content of model infant formula powders (1st age, 0–6 months), with different protein contents, generally decreased with increasing protein content. The authors attributed this to a decrease in available protein for emulsification during homogenisation as protein: fat ratio decreased, resulting in larger fat droplets, and, in some cases, increased content of free fat in the finished powder.

Mean fat globule size of model formulas in-process
FGS [D4,3] of the formulas at PM, PP, PO and PD stages is given in Table 3. FGS at PM appeared to be lower for samples with lower protein contents. After homogenisation, the FGS of all emulsions was lower, and no significant differences in FGS between samples (*P* > 0.05) were observed, that is protein content did not appear to affect oil droplet size of freshly prepared emulsions. McCarthy *et al*. (2012) demonstrated that protein:fat ratio of less than 0.26 led to decreased physical stability during subsequent processing of a 1st age model infant formula, and, hence, identified this

Table 3 Mean fat globule size of model infant formulas with protein contents of 10, 14 and 18 g/100 g and whey protein:casein ratios of 60:40 (A), 40:60 (B) and 20:80 (C) at post-mixing, post-processing, post-overnight storage and pre-drying stages.

	Post-mixing	Post-processing	Post-overnight	Pre-drying
	(μm)			
10A	3.39 ± 0.00[ab]	0.65 ± 0.00[a]	0.85 ± 0.16[a]	0.99 ± 0.29[a]
10B	3.32[ab]	0.66[a]	1.21[a]	1.42[a]
10C	3.54 ± 0.04[a]	0.69 ± 0.03[a]	0.81 ± 0.09[a]	0.84 ± 0.00[a]
14A	2.87[abc]	0.62[a]	1.31[a]	1.36[a]
14B	2.70 ± 0.05[bc]	0.64 ± 0.01[a]	1.20 ± 0.55[a]	0.88 ± 0.23[a]
14C	2.66[abc]	0.58[a]	1.63[a]	1.16[a]
18A	2.32 ± 0.10[c]	0.62 ± 0.00[a]	0.63 ± 0.01[a]	1.27 ± 0.58[a]
18B	2.12[c]	0.62[a]	0.65[a]	0.67[a]
18C	2.80 ± 0.23[abc]	0.71 ± 0.02[a]	0.74 ± 0.05[a]	0.77 ± 0.04[a]

Duplicate trials of 10A, 10C, 14B, 18A and 18C, individual trials of 10B, 14A, 14C and 18B. Total solid contents of emulsions were as follows: PM (42.5 ± 0.44 g/100 g), PP (42.1 ± 0.49 g/100 g), PO and PD (41.2 ± 1.0 g/100 g), and there was no significant difference (*P* < 0.05) between the emulsions at each stage. Values are means ± standard deviation; values within a column not sharing a common superscript differ significantly (*P* < 0.05).

ratio as the critical minimum level to ensure physical stability. The emulsions in the present study had protein:fat ratio in the range of 0.50 to 0.94 and did not appear to significantly influence emulsion stability post-homogenisation; however, results did indicate a tendency for higher levels of free fat in powders with lower protein content (i.e. lower protein:fat ratio).

During homogenisation, the adsorption of protein on the surface of the fat droplets is dependent on several factors,

including protein concentration, protein type and physical state/structure of the protein (Dickinson 1997). Previous studies have demonstrated the preferential adsorption of casein over whey proteins during homogenisation (Britten and Giroux 1991; Sourdet *et al*. 2002; Ye 2008; Liang *et al*. 2016). During homogenisation, caseins are thought to adsorb rapidly and form a thick interfacial layer on the surface of the fat droplets, thereby limiting adsorption of whey proteins. Sourdet *et al*. (2002) found that emulsions prepared at 20:80 (SMP only) or 60:40 whey protein:casein ratio (using SMP and WPI ingredients) had more protein adsorbed at the oil–water interface in comparison with emulsions prepared at 100:0 whey protein:casein ratio (WPI only). Britten and Giroux (1991) demonstrated improved emulsifying behaviour of whey proteins in heated protein solutions; the authors found that the stability of an emulsion formed by a blend of casein and whey proteins was dependent on the effect of the whey proteins increasing the viscosity of the emulsion and the effect of casein protein performance at the oil droplet interface. In contrast, for the current study, whey protein:casein ratio did not appear to have a significant effect ($P > 0.05$) on emulsion formation and stability, with the mean FGS of all formulas ranging from 0.58 to 0.71 µm at PP, 0.63 to 1.63 µm at PO and 0.67 to 1.42 µm at PD.

Viscosity of model formulas in-process

The apparent viscosity of the formulas at PM, PP, PO and PD is given in Table 4. The TS of the formulas at each stage of manufacturing were not found to be significantly different ($P > 0.05$) and ranged between 41.2 and 42.5 g/100 g. Viscosit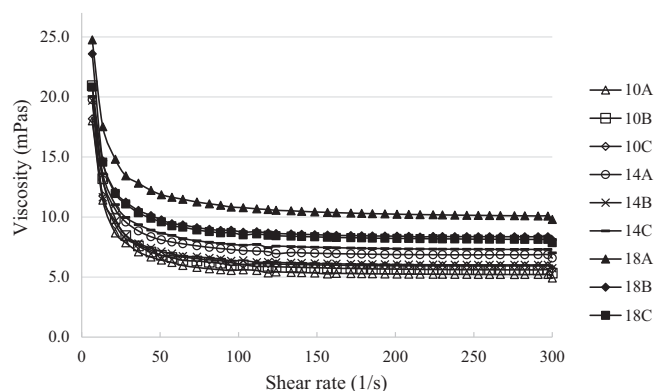y was analysed at 65 °C for samples taken at PD to best match the temperature at which the emulsions were heated prior to entering the drying chamber, while for all other stages, samples were analysed at 45 °C. The viscosity of all emulsions was found to decrease significantly ($P < 0.05$) with increasing temperature. As expected, viscosity increased significantly ($P < 0.05$) as the protein content was increased to 18 g protein per 100 g (Table 4). All formulas were found to be shear thinning at PD, with formulas containing 18 g/100 g protein having the highest viscosity on shearing at 300 s^{-1} (Figure 1). In the present study, as protein:lactose ratio increased, the viscosity of the formulas increased and it has previously been demonstrated that dairy concentrates with higher lactose contents have lower viscosity than those with higher protein contents (Westergaard 2004; Schuck *et al*. 2005; Murphy *et al*. 2018).

The influence of protein profile (whey protein:casein ratio) on viscosity at all stages of production was determined for formulas containing 14 and 18 g protein per 100 g (Table 4). Formulas at both protein concentrations with whey protein:casein ratio of 20:80 generally had significantly higher viscosity ($P < 0.05$) in comparison with formulas with the same protein content but different protein profiles (i.e. 60:40 and 40:60 whey protein:casein ratios). At equal protein concentration, casein proteins are known to contribute more than whey proteins to viscosity in solution (Marti *et al*. 2003); at concentrations greater than approximately 15% casein, hydration and swelling of caseins exponentially increased, which contributes to increased hydrodynamic volume, polymer–polymer interactions and viscosity (O'Regan *et al*. 2009). In their native state, whey proteins in solution are much less viscous than caseins at

Table 4 Apparent viscosity at 300 s^{-1} of model infant formulas with protein contents of 10, 14 and 18 g/100 g and whey protein:casein ratios of 60:40 (A), 40:60 (B) and 20:80 (C) at post-mixing, post-processing, post-overnight storage and pre-drying.

	Post-mixing	Post-processing	Post-overnight	Pre-drying
	(mPas)			
10A	6.51 ± 0.09^d	6.89 ± 0.09^d	6.18 ± 0.14^e	4.99 ± 0.07^d
10B	7.31 cd	8.12^d	6.58^{de}	5.32 cd
10C	6.87 ± 0.15 cd	8.03 ± 0.21^d	7.49 ± 0.74^{cde}	5.50 ± 0.15 cd
14A	8.08 cd	8.46 cd	8.46^{bcde}	6.58^{bc}
14B	7.97 ± 0.41^c	8.51 ± 0.25^d	8.48 ± 0.42^{cde}	5.58 ± 0.16 cd
14C	10.1^b	10.8^{bc}	10.7^{abcd}	7.02^b
18A	10.3 ± 0.07^b	11.7 ± 0.42^{ab}	11.7 ± 0.65^{ab}	9.53 ± 0.28^b
18B	10.6^{ab}	11.4^{ab}	11.2^{abc}	8.08^b
18C	12.0 ± 0.05^a	13.3 ± 0.04^a	13.1 ± 0.33^a	8.00 ± 0.14^a

Duplicate trials of 10A, 10C, 14B, 18A and 18C, individual trials of 10B, 14A, 14C and 18B. Total solid contents of emulsions were as follows: PM (42.5 $\pm$ 0.44 g/100 g), PP (42.1 $\pm$ 0.49 g/100 g), PO and PD (41.2 $\pm$ 1.0 g/100 g), and there was no significant difference ($P < 0.05$) between the emulsions at each stage. Values are means $\pm$ standard deviation; values within a column not sharing a common superscript differ significantly ($P < 0.05$).

Figure 1 Viscosity as a function of shear rate at pre drying stage for model formulas.

Table 5 Moisture, water activity, particle density and bulk density of model infant formula powders with protein contents of 10, 14 and 18 g/100 g and whey protein:casein ratios of 60:40 (A), 40:60 (B) and 20:80 (C).

	Moisture (g/100 g)	Water activity (-)	Particle density (g/mL)	Bulk density
10A	1.50 ± 0.04^b	0.15 ± 0.01^{ab}	1.10 ± 0.0^e	0.55 ± 0.02^c
10B	1.67 ± 0.03^{ab}	0.13 ± 0.00^b	1.14 ± 0.01^{abc}	0.58 ± 0.01^{ab}
10C	1.56 ± 0.07^{ab}	0.14 ± 0.00^b	1.17 ± 0.00^a	0.59 ± 0.01^a
14A	1.64 ± 0.16^{ab}	0.18 ± 0.00^{ab}	1.10 ± 0.00^{de}	0.56 ± 0.01^{bc}
14B	1.46 ± 0.16^b	0.16 ± 0.02^{ab}	1.13 ± 0.01^{bcd}	0.58 ± 0.01^{ab}
14C	1.55 ± 0.12^{ab}	0.15 ± 0.00^{ab}	1.15 ± 0.00^{abc}	0.58 ± 0.01^{ab}
18A	1.78 ± 0.11^a	0.19 ± 0.00^a	1.10 ± 0.01^e	0.56 ± 0.01^{bc}
18B	1.50 ± 0.05^{ab}	0.13 ± 0.01^b	1.12 ± 0.01^{cde}	0.56 ± 0.00^c
18C	1.78 ± 0.13^a	0.18 ± 0.02^a	1.15 ± 0.01^{ab}	0.59 ± 0.00^a

Duplicate trials of 10A, 10C, 14B, 18A and 18C, and individual trials of 10B, 14A, 14C and 18B. Values are means ± standard deviation; values within a column not sharing a common superscript differ significantly ($P < 0.05$).

the same concentration; however, when critical temperatures are exceeded, increases in viscosity are observed due to thermal denaturation of the globular structure of whey proteins (Murphy *et al*. 2014). Previous differential scanning calorimetry (DSC) studies have demonstrated that denaturation of β-lactoglobulin may initiate at temperatures >65 °C (similar to pre-heat temperature used in the present study prior to spray drying). These studies demonstrated that the denaturation temperature of β-lactoglobulin is dependent on protein purity, pH, ionic strength and protein concentration (Qi *et al*. 1995; Fitzsimons *et al*. 2007; Gotham *et al*. 2007). It is proposed that the protein concentration and the stabilising effect of the high concentration of lactose of the formulas in the present study limited denaturation on heating at 65 °C.

Physical properties of powders
No significant differences (*P > 0.05*) in the water content (1.46 to 1.78 g/100 g) and water activity (0.13 to 0.18 a_w) of the powders were identified (Table 5). Particle density of the powders ranged from 1.10 to 1.17 g/mL and bulk density from 0.55 to 0.59 g/mL (Table 5). Differences in chemical composition (i.e. protein and lactose contents) of the powders, which were deemed statistically significant, did not appear to affect the particle or bulk density. However, the particle densities for the powders with whey protein:casein ratio of 20:80 were higher (*P < 0.05*) than in powders with whey protein:casein ratio of 60:40. The calculated correlation function (0.94) indicated a negative correlation between whey protein:casein ratio and particle density of the powders. The viscosity of an emulsion immediately prior to spray drying has been linked to droplet formation, that is higher viscosity resulting in larger droplets (Rosenberg *et al*. 1990; Masters 2002; Murphy *et al*. 2013; Kelly *et al*. 2014). In the present study, significant differences in viscosity of emulsions prior to spray drying were observed for emulsions with whey protein:casein ratio of 20:80

compared with 60:40 (Table 4). It is probable that these differences in viscosity influenced the particle size, and ultimately particle and bulk density of the powders.

CONCLUSION

Protein content (10, 14 and 18 g/100 g) and whey protein: casein ratios (60:40, 40:60, 20:80) influenced the processing characteristics and physical properties of a model infant formula. After homogenisation, the FGS of all emulsions was reduced, and there were no significant differences in FGS between samples (*P > 0.05*). Viscosity of the formulas increased with increasing protein concentration, with the increases being more pronounced for higher casein content Formulas. Free fat content of the powders decreased with increasing protein content (i.e. increasing protein:fat ratio). An increase in casein concentration, which resulted in increased viscosity during processing, resulted in higher particle densities in the finished powders. Differences in protein content, protein profile and lactose content did not influence water content and water activity of the powders. This study clearly demonstrated the influence of selected protein-based formulation adjustments on processing characteristics and physical properties of infant formula powders and will support the development of infant nutritional products.

AUTHOR CONTRIBUTIONS

Emma J Walshe: Data curation, Formal analysis, Investigation, Writing-original draft, Writing-review & editing. **Jonathan O'Regan:** Methodology, Supervision, Writing-original draft, Writing-review & editing. **James A O'Mahony:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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