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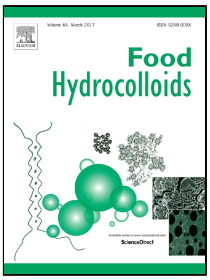

Influence of emulsifier type on the spray-drying properties of model infant formula emulsions

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Highlights:

- Emulsifier type influenced the surface composition of powders
- The formulation containing conjugated WPH had the lowest powder stickiness
- Formulations containing lipid-based emulsifiers had the highest powder stickiness
- Conjugate-stabilised emulsions had the best quality upon reconstitution

Influence of emulsifier type on the spray-drying properties of model infant formula emulsions

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Abstract

The objective of this study was to compare the drying performance and physicochemical properties of model infant formula (IF) emulsions containing 43, 96, and 192 g L^{-1} protein, oil and maltodextrin (MD), respectively, prepared using different emulsifier systems. Emulsions were stabilised using either whey protein isolate (WPI), whey protein hydrolysate (WPH; DH 8%), WPH+CITREM (9 g L^{-1}), WPH+lecithin (5 g L^{-1}) or WPH conjugated with maltodextrin (DE 12) (WPH-MD). Homogenised emulsions had 32% solids content and oil globules with mean volume diameter <1 μm. Powders were produced by spray-drying with inlet and outlet temperatures of 170 and 90°C, respectively, to an average final moisture content of 1.3%. The extent of powder build-up on the dryer wall increased in the order; WPH – MD<<WPH≤WPI<WPH+LEC≤WPH+CIT. The same trend was observed for the extent of spontaneous primary powder agglomeration, as confirmed by particle size distribution profiles and scanning electron micrographs, where the WPH-MD and WPH+CIT powders displayed the least and greatest extent of agglomeration, respectively. Analysis of elemental surface composition of the powders, showed that surface fat, protein and carbohydrate decreased in the order; WPH+CIT>WPH+LEC>WPH>WPH – MD>WPI, WPI>WPH>WPH – MD>WPH+LEC>WPH+CIT and WPH – MD>WPI>WPH>WPH+LEC>WPH+CIT, respectively. Additionally, differences in wettability, surface topography and oil globule distribution within the powder matrix and in reconstituted powders were linked to the powder emulsifier system. Inclusion of the WPH-MD conjugate in the formulation of IF powder significantly improved drying behaviour and physicochemical properties of the resultant powder, as evidenced by lowest powder build-up during drying and greatest emulsion quality on reconstitution, compared to the other model formula systems.

47 **Keywords**: Spray-dried emulsions, Infant formula powders, Protein conjugation, Powder
48 stickiness, Emulsion stability, Particle microstructure

1. Introduction

Protein-based added-value nutritional formulations have been gaining a significant share of the global food market over the last decade, especially those tailored for athletes, the elderly and infants; the total global market for these product types is predicted to exceed 100 billion USD by 2020. Formulations for such products generally contain protein (e.g., whey protein), oils rich in unsaturated fatty acids (i.e., blends of vegetable oils) and carbohydrates (e.g., maltodextrin) as the main components. Whey protein hydrolysate (WPH) is often used as a protein source in such nutritional formulae due to its desirable amino acid composition, high digestibility and rapid absorption in the gut (Hernández-Ledesma, García-Nebot, Fernández-Tomé, Amigo, & Recio, 2014). Modification of protein *via* hydrolysis has been extensively studied, with reports on improvement in protein functionality in the areas of solubility, surface activity, foaming and emulsifying properties available in the scientific literature (Agboola & Dalgleish, 1996a, b; Banach, Lin, & Lamsal, 2013; Foegeding & Davis, 2011; Kilara & Panyam, 2003). However, incorporation of WPH into nutritional formulations such as powdered formulae or ready to drink products is often associated with processing and shelf life challenges such as protein/peptide-mediated bridging flocculation and coalescence, due to reduced steric stabilisation and increased number of exposed reactive sites, compared to formulations based on intact whey protein (Drapala, Auty, Mulvihill, & O'Mahony, 2016a, b; Euston, Finnigan, & Hirst, 2000; Hunt & Dalgleish, 1995). Irrespective of the format of the final product (i.e., liquid or powder), the formulations for both physical formats have to undergo a number of thermal treatments (e.g., pasteurisation, sterilisation, spray-drying) as a liquid. Therefore, additional non-protein surface active components are often included in the formulation of WPH-based emulsions in order to improve their processing and shelf-life stability; these surfactants are usually lipid-based emulsifiers, including lecithin or citric acid esters of mono- and di-glycerides (CITREM).

Spray-drying is one of the most common processes used in the manufacture of dairy ingredients and nutritional products; rapid water removal results in increased product shelf-life, reduced shipping and storage costs and provides the consumer with a convenient and stable product. In this complex process, multiple factors such as feed characteristics (e.g., composition and rheological properties), process parameters (e.g., atomiser type and fines return) and external factors (e.g., air humidity, temperature) significantly impact the drying performance and the physicochemical properties of the final product. The composition (i.e., the type and content of protein, carbohydrate, fat and emulsifier, total solids content) and properties (i.e., flow behaviour and viscosity) of the emulsion destined for spray-drying have a strong influence on its drying properties; extensive scientific reports and reviews focusing on the effects these factors have on the characteristics and properties of the resulting powders have been published (Adhikari, Howes, Wood, & Bhandari, 2009; Jayasundera, Adhikari, Aldred, & Ghandi, 2009; Ji et al., 2016; Kim, Chen, & Pearce, 2009; Millqvist-Fureby, Elofsson, & Bergenståhl, 2001; Taneja, Ye, Jones, Archer, & Singh, 2013; Vega & Roos, 2006; Vignolles, Jeantet, Lopez, & Schuck, 2007).

It is well established that there is a strong relationship between the surface composition of powder particles and their drying performance in addition to the properties (e.g., cohesiveness, shelf-life) of the final product (Kelly, O'Mahony, Kelly, & O'Callaghan, 2014; Nijdam & Langrish, 2006; Sadek et al., 2015). In the production of fat-rich powders, high surface fat content can lead to powder stickiness, low powder recovery (i.e., yield) and production down-time (i.e., due to powder build-up on the dryer walls) as well as poor shelf life and undesirable properties of the final product (i.e., lipid oxidation, caking, low solubility and dispersibility) (Paterson, Zuo, Bronlund, & Chatterjee, 2007). Surface composition of an emulsion-based powder is governed mainly by the emulsifier system used; upon atomisation, a new air/liquid interface is created and surface active components (i.e., protein, peptides, low

99 molecular weight surfactants), present in the emulsion, migrate rapidly towards, and adsorb
100 at, the new interface, effectively reducing the surface free energy and enhancing the
101 thermodynamic stability of the system (Munoz-Ibanez et al., 2016). Effectively, surfactants
102 are over-represented at the droplet/powder particle surface, affecting in-process and in-
103 application behaviour of these products, as exhibited by interactions of particles with the
104 dryer wall and with other droplets/powder particles. Thus, a better understanding of the
105 emulsifier system and its modification to tailor it to a specific formulation has an important
106 role in increasing drying efficiency to produce a powder with desired properties.

107 Conjugation of milk proteins with carbohydrates through the Maillard reaction has been
108 frequently reported to give an emulsifier with exceptional functionality, especially with
109 respect to stability of emulsion to unfavourable thermal and/or storage conditions (Akhtar &
110 Dickinson, 2003; Drapala et al., 2016 a, b; Kasran, Cui, & Goff, 2013a, 2013b; O'Regan &
111 Mulvihill, 2010a 2010b; Wooster & Augustin, 2006). WPH-maltodextrin (WPH-MD)
112 conjugates have been shown to confer strong steric stabilisation to oil droplets, effectively
113 limiting globule-globule interactions and preventing emulsion destabilisation (i.e.,
114 flocculation and/or coalescence) (Corzo-Martínez et al., 2011; Liu, Ma, McClements, & Gao,
115 2016).

116 There is an evident potential for these conjugates to affect surface properties of spray dried
117 emulsions, effectively, influencing their behaviour during drying and properties of the final
118 product. Good interfacial barrier properties and inherent ability of WPH-MD conjugate to
119 adsorb at the newly formed air/water interface (O'Mahony, Drapala, Mulcahy, & Mulvihill,
120 2017) can offer an ingredient capable of deterring interactions between atomised emulsion
121 droplets/powder particles. However, currently there are no published studies reporting on the
122 use of WPH-based conjugates in spray dried emulsions nor on the properties of the resultant
123 powders. This study aims to directly compare the spray drying performance and powder

124 physical properties for spray dried emulsions stabilised with different emulsifier systems;
125 namely, conjugated protein/peptides (WPH), not conjugated protein/peptides (WPH, WPI)
126 and not conjugated protein/peptides (WPH) with the addition of low molecular weight lipid-
127 based surfactants (i.e., CITREM and lecithin).

128 **2. Materials and methods**

129 2.1. Materials

130 Whey protein isolate (WPI) and whey protein hydrolysate (WPH; 8% degree of hydrolysis;
131 DH) were obtained from Carbery Food Ingredients Ltd. (Ballineen, Co. Cork, Ireland). The
132 WPI and WPH ingredients had protein contents of 87.2 and 83.7%, respectively, and ash
133 contents of 2.76 and 2.92%, respectively, as reported by Drapala et al. (2016a). Maltodextrin
134 (MD) was obtained from Corcoran Chemicals Ltd. (Dublin, Ireland) and had moisture and
135 ash contents of <5.0% and <0.2%, respectively. Soybean oil was obtained from Frylite Group
136 Ltd. (Strabane, Co. Tyrone, Northern Ireland). CITREM (Grindsted® CITREM N12) was
137 obtained from Dupont Nutrition Biosciences ApS (Brabrand, Denmark) and de-oiled
138 powdered soybean lecithin (Ultralec® P) was obtained from ADM (Decatur, IL, USA). All
139 other chemicals and reagents used in the study were of analytical grade and sourced from
140 Sigma-Aldrich (Arklow, Co.Wicklow, Ireland).

141 2.2. Preparation of emulsions

142 Emulsions ($_e$) for model infant formula (IF) powders ($_p$) were prepared at pH 6.8 using
143 protein, soybean oil and maltodextrin in the ratios 1.0:2.3:4.5, respectively. The protein
144 component was either whey protein isolate (WPI), whey protein hydrolysate (WPH) or WPH
145 conjugated with maltodextrin (MD) in a wet heating process as detailed by Drapala et al.
146 (2016a). Additionally, non-protein emulsifiers, citric acid esters of mono- and di-glycerides
147 (CITREM; 9 g L^{-1}) and soybean lecithin (5 g L^{-1}) were incorporated into the formulation of

selected IF emulsions destined for subsequent spray-drying. Emulsions were prepared by dissolving oil soluble components, where applicable, in soybean oil and water soluble components in ultrapure water, followed by two stage homogenisation (double pass) at 15 and 3 MPa, using a valve homogeniser (APV GEA Niro-Soavi S.p.A., Parma, Italy) at 50°C. All emulsions were prepared to a total solids (TS) target of 32% as measured with a rapid moisture analyser (HB43 – S, Mettler – Toledo LLC, Columbus, OH, USA). In total, five emulsions based on WPI, WPH, WPH + CITREM (WPH+CIT), WPH + lecithin (WPH+LEC) and WPH conjugated with maltodextrin (WPH-MD) were produced in the current study.

2.3. Spray-drying of emulsions

Powders were produced from emulsions using a bench-top spray dryer (B-191, BÜCHI Labortechnik AG, Flawil, Switzerland) with a maximum evaporation capacity of 1.5 L H_2O h^{-1}. Inlet temperature was set at 170°C and outlet temperature was maintained at 90-95°C by controlling the aspirator power (i.e., in the range of 40-60 m^3 h^{-1}) and the feed flow rate (i.e., in the range 1.2-1.4 L h^{-1}). Effectively, drying temperatures were kept within the industry relevant range typical for IF manufacture by using high feed flow rate (95-100%) and relatively low aspirator power (80-90%); however, this was achieved at the expense of product yield (Fig. 1). The powders were collected in the collection chamber as detailed in Fig. 1, transferred to zip-sealed low density polyethylene bags (VWR International, Leuven, Belgium), followed by vacuum packing in heat-sealed polyamide/polyethylene bags (Fispak Ltd., Dublin, Ireland) with a moisture permeability of 2.6 g m^{-2}.d. The powders were stored in the dark at ambient conditions (i.e., ~20°C) until further analyses within 4 weeks of spray drying. Powder recovery was calculated on a TS basis (i.e., [Final powder product TS/feed liquid TS] ×100) from the total amount of powder obtained in the collection chamber. Losses

on drying were due to unrecoverable powder, which stuck to the wall of the dryer main chamber or fell and accumulated at the base of the main chamber during spray-drying (Fig. 1). Powder stickiness was visually assessed based on the extent of wall coating by powder in the cyclone, in order to provide information on particle cohesion arising from surface characteristics (Fig. 1).

2.4. Particle size distribution

Particle size distribution (PSD) of the emulsions immediately after homogenisation and after powder reconstitution (i.e., 12%, w/v, TS) was measured using a laser light diffraction unit (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK) equipped with a 300 RF (reverse fourier) lens, an LED light source (λ of 470 nm) and a He-Ne laser (λ of 633 nm) as detailed by Drapala et al. (2016b). The size distribution of the model infant formula powders was measured using a Mastersizer 3000 equipped with a dry powder dispenser cell (Aero S). Approximately 3.0 g of powder was placed in the feed hopper, containing a ball bearing to facilitate powder flow, with the feed pressure set at 1 bar, powder flow rate at 40-70% and the hopper height at 2 mm. All measurements were taken at 1-2% obscuration. The background and sample measurement duration was set at 20 s with the material refractive and absorption indexes of 1.46 and 0.01, respectively.

2.5. Rheological measurements

The apparent viscosity of emulsions was measured at 20°C using a rotational viscometer (Haake RotoVisco 1, Thermo Fisher Scientific, MA, USA) equipped with a cylindrical double gap cup and rotor (DG43, Thermo Fisher Scientific, MA, USA) as described by Mulcahy, Mulvihill and O'Mahony (2016). The shear rate was increased from 0 to 300 s^{-1} over 5 min, held at 300 s^{-1} for 2 min and decreased to 0 s^{-1} over 5 min; the average apparent viscosity was determined at 300 s^{-1} (η_{300}) for each emulsion. The power law of shear stress (τ) versus shear rate (γ) was used to obtain flow curves and the flow behaviour parameters

consistency coefficient (K) and flow behaviour index (n) as detailed by Anema, Lowe, Lee, and Klostermeyer (2014). The flow behaviour index (n) values are used to describe the flow behaviour of liquid samples where $n < 1$, $n > 1$ and $n = 1$ indicate shear-thinning, shear-thickening and Newtonian flow behaviour, respectively.

2.6. Composition and colour analyses of powders

The chemical composition of the model infant formula powders was determined using standard International Dairy Federation (IDF) methods as detailed by Drapala, Auty, Mulvihill, and O'Mahony (2015). Colour of the powders was measured using a pre-calibrated colorimeter (Minolta Chroma Meter CR-400, Minolta Ltd., Milton Keynes, U.K.) equipped with a granular-materials attachment CR-A50. Colour was expressed using the Commission Internationale de l'Eclairage (CIE) colour chromaticity L* a* b* scale (L = dark/light, a = red/green, b = yellow/blue).

2.7. Powder wettability

The sessile drop goniometric method was used to determine the wettability of powders. All powders were compressed for 10 s at 78.4 MPa using a manual press (15 ton Manual Hydraulic Press, Specac Ltd., Orpington, UK) to form pellets (13 mm diameter); all pellets had a density of 1.08 (± 0.05) g cm^{-3}. Subsequently, the mean contact angle (θ) was determined directly using an optical tensiometer (Attension Theta, Biolin Scientific, Stockholm, Sweden); a drop (10 µl) of ultrapure water was formed and deposited on top of a powder pellet and the reduction in contact angle during the first 30 s was recorded using a high-resolution digital camera (15 frames per second) and processed using image analysis software (OneAttension, Biolin Scientific).

2.8. Surface composition of powders

Surface free fat content of powders was determined using the GEA Niro analytical method (GEA Niro, 2005) as described by McCarthy et al. (2013) with modified quantities of powder (5.0 g), petroleum ether (30 mL) and filtrate (15 mL) used. Elemental composition of powder surfaces was determined by X-ray photoelectron spectroscopy (XPS; Kratos Axis 165, Kratos Analytical, UK) as detailed by McCarthy et al. (2013). A matrix formula was used to calculate relative amounts of protein, fat and carbohydrate on the powder surface, as detailed by Fäldt, Bergenståhl, and Carlsson (1993).

2.9. Microstructure of powders

2.9.1. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) analysis of powder particles was performed using a confocal laser scanning microscope (TCS SP, Leica Microsystems CMS GmbH, Wetzlar, Germany). Powders were deposited onto a glass slide and excess sample was removed with compressed air. The powder samples were stained with a mixture (3:1) of Nile Red (0.10 g L^{-1} in polyethylene glycol) and Fast Green (0.01 g L^{-1} in water) fluorescent dyes (Sigma Aldrich, Wicklow, Ireland) to label the fat and protein components of the powders, respectively. Visualisation of oil and protein in the powders was carried out using an Ar laser (excitation = 488 nm, emission = 500-530 nm) and He – Ne laser (excitation = 633 nm, emission = 650-700 nm), respectively. At least 3 representative images of each sample were taken using 63 × oil immersion objective.

2.9.2. Scanning electron microscopy

Scanning electron microscopy (SEM) analysis of powders was performed using a scanning electron microscope (JSM – 5510, Jeol Ltd., Tokyo, Japan). Samples were mounted on double-sided carbon tape, attached to SEM stubs, and then sputter-coated with gold/palladium (10 nm; Emitech K550X, Ashford, UK). Representative micrographs were

taken at 5 kV at 1000 × (i.e., overview of powder population) and 3000 × (i.e., shape and surface topography of powder particles) magnifications. At least three specimens of each sample were observed to obtain representative micrographs of samples.

2.10. Statistical data analysis

All powders were prepared in three independent trials and all measurements were carried out in at least duplicate. Analysis of variance (ANOVA) was carried out using the Minitab® 16 (Minitab Ltd., Coventry, UK, 2010) statistical analysis package. The Tuckey method was used to obtain grouping information. The level of significance was determined at $P < 0.05$.

3. Results

3.1. Emulsion characteristics

The emulsions had TS levels ranging from 32.2 to 32.7% prior to spray-drying (Table 1). Particle size analysis showed that all emulsions had oil globules with mean volume diameters ($D_{4,3}$) less than 1 μm and no statistically-significant differences in $D_{4,3}$ were found between the emulsions (Table 1). Similarly, no significant differences in the apparent viscosity (η_{300}) were observed between WPI_e, WPH_e, $WPH+CIT_e$ and $WPH+LEC_e$ emulsions; however, the η_{300} for the $WPH - MD_e$ emulsion was significantly lower than that of the WPI_e, and $WPH+CIT_e$ emulsions (Table 1). Analysis of the flow behaviour showed no significant differences between emulsions, where most emulsions displayed a shear-thinning behaviour (i.e., $n < 1$) (Table 1). A reduction in the viscosity during shearing (i.e., shear-thinning) of protein solutions is, generally, a result of spatial rearrangement of protein molecules in the liquid and of disruptions in their steady-state interactions (Walstra, Wouters, & Geurts, 2006); in emulsions, shear-thinning can be associated with flocculation of oil droplets (Xu, Wang, Jiang, Yuan, & Gao, 2012). Additionally, in a concentrated emulsion system (i.e., TS = 32%), packing of oil globules is denser than in a dilute emulsion (i.e., TS ≤ 12%) and

268 interactions between its constituents, as monitored by flow behaviour analysis, can be also
269 related to physical contact between molecules located at the interfaces of oil globules
270 (O'Mahony, et al., 2017). The formation of ternary complexes between unadsorbed
271 protein/peptides, CITREM and maltodextrin (Drapala et al., 2016b; Semenova, Myasoedova,
272 & Antipova, 2001) in the WPH+CIT_e emulsion, or the presence of intact whey protein in the
273 serum phase and at the interfaces of oil globules in the WPI_e emulsion, is likely to have
274 contributed to higher viscosity of these emulsions, compared to the other samples.

275 3.2. Drying performance

276 Fig. 2 illustrates differences in drying behaviour between liquid concentrates/powders as
277 evidenced by different levels of wall-coating (i.e., multilayer particle cohesion) by fine
278 powder particles in the cyclone of the spray dryer. The extent of this coating is assumed to be
279 directly related to powder stickiness; the observed stickiness can be divided into 3 groups
280 based on the level of coating, i.e., non-sticky (negligible coating), moderately sticky (partial
281 coating) and very sticky (complete coating) (Fig. 2; Table 3). Using this classification, the
282 WPI_p and WPH_p powders were moderately sticky, WPH+CIT_p and WPH+LEC_p powders
283 were very sticky and the WPH-MD_p powder was non − sticky.

284 Differences in the stickiness of powders had a direct impact on the powder recovery (i.e.,
285 product yield; Table 3); the recovery of product was lower for products with higher level of
286 stickiness. Powders containing non-protein emulsifiers (WPH+LEC_p and WPH+CIT_p)
287 displayed the lowest powder recovery (18.1 and 21.3%, respectively) followed by WPI_p
288 (22.0%), WPH_p (26.1%) and WPH-MD_p (55.3%). It should be noted that in order to facilitate
289 the use of industry-relevant drying temperatures (i.e., 170°C and 90-95°C for inlet and outlet,
290 respectively) high feed flow rate (95-100%) and relatively low aspirator power (80-90%)
291 conditions were used. These conditions caused deposition of higher-moisture particles at the
292 periphery of the atomised feed jet on the inner wall of the main drying chamber (Fig. 1) and

293 contributed to the low powder yield. Sticking of powders to the inner wall of a spray dryer is
294 a common challenge in industry and it directly affects the product yield and drying efficiency
295 (i.e., cleaning and down-time). In high-fat powders (e.g., infant formulae) stickiness is
296 strongly related to the powder surface composition, while in low-fat, protein-dominant
297 powders, it is generally related to the efficiency of water removal and glass transition
298 properties of the system (Kelly et al., 2014). Generally, the more fat at the powder surface the
299 greater the challenges with powder stickiness (Sharma, Jana, & Chavan, 2012; Paterson et al.,
300 2007).

301 The highest levels of stickiness in this study were observed for powders containing lipid-
302 based emulsifiers (CITREM and lecithin) while the powder containing the protein-based
303 conjugate displayed the lowest stickiness. The physicochemical characteristics of CITREM
304 and lecithin have directly affected cohesiveness (i.e., stickiness) of powders; their high
305 mobility and surface activity facilitates rapid migration to the surface of emulsion droplets
306 formed on atomisation and their relatively low melting temperatures (55-65°C) make them
307 plastic and adhesive under the environmental conditions of spray-drying. Similarly, the
308 surface active WPH – MD conjugate can also rapidly move to and adsorb at the surface of
309 atomised droplets (O'Mahony et al., 2017).

310 3.3. Powder analyses

311 3.3.1. Composition and colour of powders

312 Compositional analysis of powders showed that the measured levels (Table 2) were in line
313 with the target levels for all samples (i.e., 12.1 – 12.7% protein, 26.9 – 29.0% fat and 56.1 –
314 58.8% carbohydrate). No significant differences were found in the fat, carbohydrate or
315 moisture content between the powders. No significant differences in colour were found
316 between WPI_p, WPH_p and $WPH+CIT_p$ powders; these powders had high L* and low b*
317 values compared to the $WPH-MD_p$ and $WPH+LEC_p$ powders (Table 2). These differences

were most likely due to the presence of melanoidins (conjugation products) and carotenoids (naturally present in lecithin) in the WPH-MD_p and WPH+LEC_p powders, respectively (Liu, Ru, & Ding, 2012; McSweeney, 2008; Scholfield, 1981) as previously reported by Drapala et al. (2016b).

3.3.2. Particle size distribution of powders

All powders had relatively small particles (i.e., $D_{4,3}$ of 14.2 – 41.1 μm; Table 3). The biggest particles were observed for the WPH+LEC_p, followed by the WPH+CIT_p, WPI_p, WPH_p and WPH-MD_p powders (Table 3, Fig. 3B). In addition, powders containing lipid-based surfactants, WPH+LEC_p and WPH+CIT_p, had a distinct shoulder on the higher end (i.e., at ~100 μm) of the size range, with a notable proportion of the particle population (i.e., 7.78 and 4.05%, respectively) in these powders having diameter >100 μm (Fig. 3B; Table 3). A much smaller shoulder was also present in the WPI_p and smaller still in the WPH_p powders (i.e., 2.93 and 2.26% of particle population were >100 μm, respectively). The WPH-MD_p powder had a monomodal profile with the narrowest size distribution, where the majority (i.e., ~99%) of particles had diameters <40 μm (Fig. 3B); this sample also had the largest proportion of fine particles (i.e., 19.9% of total population had diameter <5 μm; Table 3). The greater proportion of small particles in the WPH-MD_p powder, compared to the other powders is likely related to this liquid concentrate feed having the lowest viscosity of all samples (Pisecky, 2012). Relationship between feed viscosity and the size of particles in the resultant powder was also reported by Crowley, Gazi, Kelly, Huppertz, and O'Mahony (2014), where increase in the particle size followed the increase in feed viscosity.

3.3.3. Powder wettability

The results for contact angle (θ) analysis showed that the highest θ was observed for WPH+CIT_p, followed by WPI_p > WPH+LEC_p > WPH-MD_p > WPH_p (Table 3). Generally, the more hydrophobic the surface (i.e., surface of powder pellet), the lower is its affinity for

interactions with water and, effectively, the higher the θ between the droplet of water placed on that surface. Thus, the contact angle analysis is often used to study the affinity of powders for interactions with water, providing information on powder wettability (i.e., lower θ = better wettability). The differences in wettability between the WPI_p and WPH_p powders, evidenced by different θ, were most likely directly related to differences in the physical state of protein (i.e., native *vs* hydrolysed, respectively). Solubility is generally enhanced by protein hydrolysis due to partial disruption of protein secondary and tertiary structure resulting in increased water access and faster hydration in hydrolysed, compared with intact, protein-based powders (Banach et al., 2013; Chobert, Bertrand-Harb, & Nicolas, 1988; Kelly, O'Mahony, Kelly, & O'Callaghan, 2016; Panyam & Kilara, 1996). Longer wettability times for model infant formula powders based on intact whey protein compared to partially hydrolysed whey protein were reported previously by Murphy et al. (2015). Wettability of the WPH-MD_p was similar to that observed for the WPH_p (Table 3). The better powder wettability observed for the WPH+LEC_p, compared to the WPH+CIT_p, was likely due to the differences in the nature of the two surfactants; CITREM and lecithin are anionic and zwitterionic (i.e., amphoteric) surfactants, respectively (McSweeney 2008). Lecithin is often coated onto the surface of the powders in a fluidised bed to facilitate improved solubility (i.e., instantisation) (Hammes, Englert, Zapata Norena, & Medeiros Cardozo, 2015).

3.3.4. Surface composition of powders

No significant differences were found in the free fat content for all powders due to large standard deviations, especially observed for the WPH+LEC_p powder (Table 3). A trend was observed, where free fat content was generally higher, for the WPH+CIT_p, WPH_p and WPH+LEC_p powders (i.e., 20.0, 22.9 and 25.4%, w/w, free fat, respectively), compared to the WPH-MD_p and WPI_p powders (i.e., 13.3 and 14.1%, w/w, free fat, respectively).

Table 3 shows differences in the surface composition (i.e., as measured using XPS) between the spray-dried model IF powders prepared in this study. The level of protein at the surface was highest for the WPI_p powder followed by WPH_p, $WPH\text{-}MD_p$, $WPH+LEC_p$ and $WPH+CIT_p$ powders. The highest levels of surface fat were found in the $WPH+CIT_p$ and $WPH+LEC_p$ powders. The amount of carbohydrate present at the surface was significantly higher for the $WPH\text{-}MD_p$ powder compared to the 2 powders containing lipid-based surfactants (i.e., $WPH+LEC_p$ and $WPH+CIT_p$).

The differences between the surface fat composition as measured by the solvent extraction and by the XPS methods can be explained by the different principles underpinning these methods. For the solvent extraction method the results are presented as the weight of extractable fat as a % of the powder sample weight; conversely in the XPS method, the results are presented as the % of surface area of the powder particle occupied by fat. For the XPS method only a 10 nm depth of the surface of the powder particle is analysed (Kim, Chen, & Pearce, 2009). Conversely, the solvent extraction approach extracts fat present at the surface of the powder particle as well as fat present at other locations within its interior. According to a model proposed by Buma (1971) the solvent-extractable free fat for dairy powders consists of surface fat, outer layer fat from fat globules within the surface layer of the particle, capillary fat constituted by fat globules that can be reached by the solvent through capillary forces, and dissolution fat consisting of fat reached by solvent through holes left by already extracted fat. A range of solvent extraction-based methods for assessment of the amount of free or surface fat in spray-dried emulsions, reported in the scientific literature, were compiled by Roos and Vega (2006) and it was shown that a these methods use different solvent types (petroleum ether, hexane, pentane and carbon tetrachloride) solvent-to-powder ratios (5:1 – 40:1) and powder-solvent contact times (30 s – 48 h). The solvent extraction method used in this study (GEA Niro, 2005) for quantification of the surface free fat in the

392 milk powders, with an extraction time of 15 min, could have led to the extraction of lipid
393 material in addition to surface fat alone (i.e., fat from the surface and from the interior of the
394 powder particles).

395 3.3.5. Microstructure of powders

396 3.3.5.1. Scanning electron microscopy

397 Fig. 4 A and B illustrate the detailed morphology (shape and structure) of the spray-dried
398 model IF powders. Differences between samples were mainly manifested by the extent of
399 particle agglomeration (i.e., spontaneous agglomeration of primary particles) and the
400 topography of the particle surfaces in the powders. Powders containing lipid-based
401 emulsifiers, WPH+CIT$_p$ and WPH+LEC$_p$, displayed the greatest extent of particle
402 agglomeration, followed by WPI$_p$, WPH$_p$ and WPH-MD$_p$ (Fig. 4A). Such agglomeration is
403 generally caused by extensive particle cohesion (i.e., sticking) and is evidenced by the
404 presence of 'bunch of grape'-type agglomerates (Pisecky, 2012), as observed in this study for
405 the WPH+CIT$_p$, WPH+LEC$_p$ and, to a lesser extent, WPI$_p$ powders (Fig. 4A).). These
406 observations closely match the particle size distribution data discussed in Section 3.3.2. and
407 indicate cohesive interactions between particles during spray-drying.

408 The surface topography was also different between the powders; smooth surfaces were
409 observed for the WPI$_p$ and to a lesser extent for WPH-MD$_p$ while the powder particles in the
410 WPH$_p$, WPH+CIT$_p$ and WPH+LEC$_p$ had an uneven surface with numerous bumps (WPH$_p$) or
411 craters (WPH+CIT$_p$ and WPH+LEC$_p$) present on the surface (Fig. 4B). The presence of crater
412 – like structures on the surface of spray – dried emulsions/powders has been associated with
413 broken oil globules resulting in high levels of surface fat (Drusch & Berg, 2008).
414 Additionally, WPH – MD$_p$ powder particles appeared to be partially collapsed (i.e.,
415 shrivelled) unlike particles in the other powders. Such shrivelled/buckled structures in spray-
416 dried powders has been linked with temperature-dependent changes in the volume of

417 occluded air (i.e., inflation followed by deflation of intra-particle air as the particle moves
418 from hot toward the cooler regions of the dryer) (Walton & Mumford, 1999) and with the
419 mechanical properties of the skin layer of the drying particles (Sadek et al., 2015, 2016).

420 3.3.5.1. Confocal laser scanning microscopy

421 Powders produced in the current study had generally similar particle structures, where
422 individual oil droplets were homogenously distributed within a protein-carbohydrate network
423 (Fig. 4C). The only exception was the WPH_p powder, where the oil phase appeared to be
424 largely present as irregular and extensive oil pools. Differences in the size of oil droplets
425 within the powder matrix were observed; powders containing lipid-based surfactants,
426 $WPH+CIT_p$ and $WPH+LEC_p$ had markedly bigger (2-3 μm) oil droplets embedded in the
427 powder structure, compared to apparently smaller (≤ 1 μm) oil droplets in the WPI_p and
428 $WPH-MD_p$ powders. Pools of oil or large oil droplets observed in CLSM micrographs can be
429 related to poor stability of these emulsions to processing. Additionally, 'empty' regions were
430 observed in the centre of the $WPH-MD_p$ powder (Fig. 4C); these regions most likely indicate
431 the presence of internal air pockets (i.e., vacuoles) in particles of this powder as discussed in
432 Section 3.3.5.1. Formation of vacuoles and shrivelling of powder particles have been shown
433 to take place concomitantly (Sadek et al., 2015) and is strongly linked to the surface
434 composition of the droplet and, effectively, its drying kinetics (Nijdam & Langrish, 2006;
435 Vignolles et al., 2007).

436 3.3.6. Particle size distribution after reconstitution of powders

437 Notable differences were observed in the PSD between the reconstituted IF powders (Table
438 3; Fig. 3C); the mean volume diameter ($D_{4,3}$) and the value for the 90% quantile of the size
439 distribution ($D_{v,0.9}$) were higher for all reconstituted powders compared to the emulsions prior
440 to spray drying (Tables 1 and 3; Fig. 3A and C). The observed increases in $D_{4,3}$ and $D_{v,0.9}$
441 were most pronounced for the WPH_p and $WPH+CIT_p$ powders (i.e., increases in $D_{4,3}$ and

$D_{v,0.9}$ to ≥ 5 μm and >13 μm, respectively); only a limited increase was observed for the WPH-MD_p powder (i.e., $D_{4,3}$ < 1 μm and $D_{v,0.9}$ < 2 μm) (Table 3). The $D_{4,3}$ and $D_{v,0.9}$ parameters are particularly sensitive to changes at the large particle periphery of the size distribution and their increase can be used as an indicator of associations between the larger components in a system (i.e., coalescence and/or flocculation of oil globules in this case).

These differences reflect different stabilities of the corresponding formulations to the spray-drying conditions (i.e., stability of oil globules against coalescence in a concentrated emulsion system and stability to high heat and high shear stress in the atomiser chamber and upon atomisation) and support the CLSM observations (see Section 3.3.5.1).

4. Discussion

The stability of emulsions to spray-drying was different for the studied formulations, as illustrated by the size distribution of oil globules in the powder matrix and in the reconstituted emulsions. These differences can be explained by the properties of the emulsifier systems used in these formulations, and their effect on stabilising emulsions against globule coalescence or heat-induced flocculation during processing. During spray-drying, emulsion-based systems are subjected to considerable stresses which can cause protein aggregation, breaking and coalescence of oil globules; this can lead to high surface free fat content and, effectively, undesirable properties of the resultant powder. Emulsions stabilised by high molecular weight (M_w) surfactants (e.g., protein) usually have thick and elastic interfacial films and are more stable to stress, compared to those stabilised by low M_w surfactants (e.g., CITREM, lecithin), which are prone to coalescence when forced in a close contact (Taneja et al., 2013). Formulations based on WPH often display poor thermal stability, due to exposure of reactive sites (e.g., free sulphydryl groups) at the surfaces of oil globules and in the bulk phase, often resulting in bridging flocculation of oil globules (Agboola, Singh, Munro, Dalgleish, & Singh, 1998; Drapala et al., 2016a). Such behaviour

467 was also reported in the current study, where oil pools in the WPH_p powder matrix and large
468 oil globules in this powder after reconstitution were present.

469 CITREM and lecithin are often added to improve thermal stability of WPH-based emulsions;
470 however, their presence can lead to competitive destabilisation, where protein/peptide-based
471 surfactants are displaced from the interfaces by smaller surfactants, promoting coalescence of
472 oil globules (Drapala et al., 2016a; Kaltsa, Paximada, Mandala, & Scholten, 2014; Mackie,
473 Gunning, Wilde, & Morris, 1999; Van Aken, 2003; Wilde, Mackie, Husband, Gunning, &
474 Morris, 2004). This was observed in the current study for CITREM- and lecithin-containing
475 powders, where large oil globules were observed in the powder matrix and in the
476 reconstituted emulsions (Fig. 4C, Table 3). In addition, topographical features observed for
477 samples containing lipid-based emulsifiers (i.e., craters; Fig. 4B) indicated that coalescence
478 of oil globules resulted in the presence of damaged oil globules at the powder surface
479 (Drusch & Berg, 2008). It is generally accepted that strong steric stabilisation of oil globules,
480 provided by protein-carbohydrate conjugates, can greatly limit these forms of destabilisation
481 (O'Mahony et al., 2017; Oliver, Melton, & Stanley, 2006). The presence of WPH-MD
482 conjugate in emulsions prevents interactions between individual oil globules and interactions
483 with bulk protein/peptides, resulting in enhanced stability. Results presented in the current
484 study show that superior stability of emulsions to spray-drying was achieved when the WPH-
485 MD conjugate was present in the formulation, compared to formulations containing CITREM
486 or lecithin.

487 In an emulsion, surface active molecules (e.g., protein, peptides, lecithin, CITREM,
488 conjugates) are adsorbed at the oil/water interface, where they stabilise oil globules; these
489 compounds are, generally, also abundant in the emulsion bulk phase as they are present in
490 excess of the concentration required for oil stabilisation. Upon atomisation, a new interface
491 (water/air) is formed at the surface of the atomised droplets and, during very short time

492 scales, surface active components move from the bulk to this new surface, adsorb and
493 rearrange (Munoz-Ibanez et al., 2016). Smaller surfactants move and adsorb faster due to
494 their higher mobility compared to large surfactants (Landstrom, Alsins, & Bergenstahl,
495 2000). Similar to the stabilisation of oil globules, the composition and structure of interfacial
496 layer of atomised droplets dictate their potential for interactions (i.e., stickiness,
497 agglomeration) (Nijdam & Langrish, 2006); in effect, surface composition and
498 physicochemical properties of the resulting powder are largely dependent on the surfactant
499 system of the emulsion. The high surface fat level observed for the WPH+CIT_p and
500 WPH+LEC_p powders and the high surface maltodextrin level observed for the WPH-MD_p
501 powder, could indicate preferential adsorption of lipid-based and conjugate-based
502 emulsifiers, respectively, at the surfaces of atomised droplets in these powders. Owing to the
503 different surface compositions, powders displayed different propensity for interactions
504 between individual atomised droplets/particles (i.e., primary spontaneous agglomeration) and
505 with the wall of the spray dryer (as measured by powder build-up in the cyclone). It is
506 generally recognised that high levels of surface free fat cause challenges with cohesive
507 interactions of powders (Jayasundera et al., 2009; Vega & Roos, 2006). Similarly, in the
508 current study, the likely preferential presence of lipid-based emulsifiers on the surface of
509 some of the powders may have contributed to greater cohesiveness and, effectively, could
510 have promoted agglomeration and powder build-up, compared to the other powders.

511 Properties of the feed and drying kinetics generally govern the shape of powder particles
512 (Walton & Mumford, 1999). Distinctive shrivelled particles observed for the WPH-MD_p
513 powder were likely related to significantly lower viscosity of that emulsion, compared to the
514 other emulsions (i.e., at the same TS content), effectively, impacting the rate of water
515 removal. Additionally, the more hydrophilic nature of the surface of atomised
516 droplets/powder particles for the WPH-MD_p system, resulting from higher surface

maltodextrin content, compared to the other samples could have promoted faster water removal as evidenced by the lower moisture content of the resultant powder. According to a study by Sheu and Rosenberg (1998), surface indentation for whey protein-based powders was promoted by high drying rates, leading to wall solidification before the onset of particle inflation. With progressive water removal during drying of a dairy-based system, a skin layer is formed at the droplet surface and its properties further affect the kinetics of drying and the final shape of the dried particles. Sadek et al. (2015) presented a model for mechanical properties of skin layer of a droplet during drying, where, depending on protein type present at the surface (i.e., whey protein or micellar casein), the mechanical properties of the skin were different and affected the shape of the resultant dried particles. Those authors showed that in casein micelle-dominant skins, the elastic modulus increased faster and the protein skin reached the plasticity region earlier, producing shrivelled particles with ductile and plastic skin, while it took longer for the whey protein-dominant skin to reach the plasticity region, giving round particles with brittle and plastic skins. Particle indentation for whey protein-based powders was reported to be linked to the ratio of protein to maltodextrin at the surface of powder particles (Rosenberg & Young, 1993; Sheu & Rosenberg, 1998), where surface indentation was inversely related to the proportion of whey protein in the particle skin. In the study by Sheu and Rosenberg (1998), the authors showed that increasing the maltodextrin proportion in the skin decreased its elasticity and, effectively led to the formation of shrivelled powder particles. Such shrivelled morphology was observed in this study for the WPH-MD_p powder particles. In addition, the presence of vacuoles observed in the WPH-MD_p powder sample supports its fit to the model proposed by Sadek et al. (2015), where vacuole formation and particle shrivelling were concomitant. With rapid water removal from the atomised droplets during spray-drying, less latent heat energy is required due to lower moisture content, and the energy (i.e., temperature) acting on the non-water

powder components is increased. This, effectively, can result in increased inflation of the droplet due to the expanding volume of air occluded within, followed by particle collapse (i.e., deflation) as the particles moves away from the heat source, resulting in a shrivelled hollow powder particle (Hecht & King, 2000; Walton & Mumford, 1999). The use of different emulsifier systems resulted in different surface composition of the resultant powders as well as different quality of reconstituted emulsions. It was demonstrated that the differences in powder surface composition influenced the kinetics of drying for these formulations and governed the cohesive interactions between atomised droplets/powder particles. Effectively, the presence of lipid-based emulsifiers (i.e., CITREM or lecithin) in formulations greatly increased the cohesive interactions resulting in extensive spontaneous primary agglomeration and, effectively, reduced product yield. On the other hand, when the conjugate-based emulsifier was present in the formulation, these cohesive interactions were markedly reduced.

5. Conclusions

The current study demonstrated that using the WPH-MD conjugate in the formulation of emulsion-based model IF powder improved its processing stability and affected the surface composition of resultant powder. The use of the conjugate in the formulation gave powder with decreased surface fat and increased surface carbohydrate levels, compared to systems containing lipid-based emulsifiers (i.e., CITREM or lecithin). In effect, conjugate-based powders displayed reduced cohesive behaviour, resulting in decreased agglomeration and markedly higher product yield; the opposite was observed for the powders containing lipid-based emulsifiers. This study showed that the surface composition of an emulsion-based powder and, effectively, its drying performance and final product characteristics were greatly improved by utilisation of interactions between the two components of the formulation (i.e., protein and carbohydrate). A significant potential was accentuated for conjugate-based

emulsifiers for applications in emulsion-based powders, where powder cohesion is a challenge.

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References

Adhikari, B., Howes, T., Wood, B. J., & Bhandari, B. R. (2009). The effect of low molecular weight surfactants and proteins on surface stickiness of sucrose during powder formation through spray drying. *Journal of Food Engineering, 94*, 135-143.

Agboola, S. O., & Dalgleish, D. G. (1996a). Enzymatic Hydrolysis of Milk Proteins Used for Emulsion Formation . 1 . Kinetics of Protein Breakdown and Storage Stability of the Emulsions, *Journal of Agricultural and Food Chemistry, 44*, 3631-3636.

Agboola, S. O., & Dalgleish, D. G. (1996b). Enzymatic Hydrolysis of Milk Proteins Used for Emulsion Formation. 2. Effects of Calcium, pH, and Ethanol on the Stability of the Emulsions. *Journal of Agricultural and Food Chemistry, 44*, 3637-3642.

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

Akhtar, M., & Dickinson, E. (2003). Emulsifying properties of whey protein–dextran conjugates at low pH and different salt concentrations. *Colloids and Surfaces B: Biointerfaces, 31*, 125-132.

Anema, S. G., Lowe, E. K., Lee, S. K., & Klostermeyer, H. (2014). Effect of the pH of skim milk at heating on milk concentrate viscosity. *International Dairy Journal, 39*, 336-343.

Banach, J. C., Lin, Z., & Lamsal, B. P. (2013). Enzymatic modification of milk protein concentrate and characterization of resulting functional properties. *LWT - Food Science and Technology, 54*, 397-403.

Buma, T. J. (1971). Free fat in spray dried whole milk 4. Significance of free fat for other properties of practical importance. *Netherlands Milk and Dairy Journal*. *25*, 88-106.

Chobert, J. M., Bertrand-Harb, C., & Nicolas, M. G. (1988). Solubility and Emulsifying Properties of Caseins and Whey Proteins Modified Enzymatically by Trypsin. *Journal of Agricultural and Food Chemistry, 36*, 883-892.

Corzo-Martínez, M., Soria, A. C., Villamiel, M., Olano, A., Harte, F. M., & Moreno, F. J. (2011). Effect of glycation on sodium caseinate-stabilized emulsions obtained by ultrasound. *Journal of Dairy Science, 94*, 51-58.

Crowley, S. V, Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A. (2014). Influence of protein concentration on the physical characteristics and flow properties of milk protein concentrate powders. *Journal of Food Engineering, 135*, 31-38.

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., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2016a). Improving thermal stability of hydrolysed whey protein-based infant formula emulsions by protein–carbohydrate conjugation. *Food Research International, in-press*, http://doi.org/10.1016/j.foodres.2016.01.028

Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2016b). Performance of whey protein hydrolysate-maltodextrin conjugates as emulsifiers in model infant formula emulsions. *International Dairy Journal, 62*, 76-83.

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray-drying: Localisation, determination and impact on oxidative stability. *Food Chemistry, 109*, 17-24.

Euston, S. R., Finnigan, S. R., & Hirst, R. L. (2000). Aggregation kinetics of heated whey protein-stabilized emulsions. *Food Hydrocolloids, 14*, 155-161.

624 Faldt, P., Bergenstahl, B., & Carlsson, G. (1993). The surface coverage of fat on food
625 powders analyzed by ESCA (electron spectroscopy for chemical analysis). *Food Structure,*
626 *12*, 225-234.

627 Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive
628 approach. *Food Hydrocolloids, 25*, 1853-1864.

629 Hammes, M. V, Englert, A. H., Zapata Norena, C. P., & Medeiros Cordozo, N. S. (2015).
630 Study of the influence of soy lecithin addition on the wettability of buffalo milk powder
631 obtained by spray-drying. *Powder Technology, 277*, 237-243.

632 Hecht, J. P., & King, C. J. (2000). Spray drying: influence of developing drop morphology on
633 drying rates and retention of volatile substances. 1. Single-drop experiments. *Industrial &*
634 *Engineering Chemistry Research, 39*, 1756-1765.

635 Hernández-Ledesma, B., García-Nebot, M. J., Fernández-Tomé, S., Amigo, L., & Recio, I.
636 (2014). Dairy protein hydrolysates: Peptides for health benefits. *International Dairy Journal,*
637 *38*, 82-100.

638 Hunt, J. A., & Dalgleish, D. G. (1995). Heat Stability of Oil-in-Water Emulsions Containing
639 Milk Proteins: Effect of Ionic Strength and pH. *Journal of Food Science, 60*, 1120-1123.

640 Jayasundera, M., Adhikari, B., Aldred, P., & Ghandi, A. (2009). Surface modification of
641 spray dried food and emulsion powders with surface-active proteins: A review. *Journal of*
642 *Food Engineering, 93*, 266-277.

643 Ji, J., Fitzpatrick, J., Cronin, K., Maguire, P., Zhang, H., & Miao, S. (2016). Rehydration
644 behaviours of high protein dairy powders: The influence of agglomeration on wettability,
645 dispersibility and solubility. *Food Hydrocolloids, 58*, 194-203.

646 Kaltsa, O., Paximada, P., Mandala, I., & Scholten, E. (2014). Physical characteristics of
647 submicron emulsions upon partial displacement of whey protein by a small molecular weight
648 surfactant and pectin addition. *Food Research International, 66*, 401-408.

649 Kasran, M., Cui, S. W., & Goff, H. D. (2013a). Covalent attachment of fenugreek gum to soy
650 whey protein isolate through natural Maillard reaction for improved emulsion stability. *Food*
651 *Hydrocolloids, 30*, 552-558.

652 Kasran, M., Cui, S. W., & Goff, H. D. (2013b). Emulsifying properties of soy whey protein
653 isolate-fenugreek gum conjugates in oil-in-water emulsion model system. *Food*
654 *Hydrocolloids, 30*, 691-697.

655 Kelly, G. M., O'Mahony, J. A., Kelly, A. L., & O'Callaghan, D. J. (2014). Physical
656 characteristics of spray-dried dairy powders containing different vegetable oils. *Journal of*
657 *Food Engineering, 122*, 122-129.

658 Kelly, G. M., O'Mahony, J. A., Kelly, A. L., & O'Callaghan, D. J. (2016). Water sorption
659 and diffusion properties of spray-dried dairy powders containing intact and hydrolysed whey
660 protein. *LWT - Food Science and Technology, 68*, 119-126.

661 Kilara, A., & Panyam, D. (2003). Peptides from milk proteins and their properties. *Critical*
662 *Reviews in Food Science and Nutrition, 43*, 607-633.

663 Kim, E. H. J., Chen, X. D., & Pearce, D. (2009). Surface composition of industrial spray-
664 dried milk powders. 1. Development of surface composition during manufacture. *Journal of*
665 *Food Engineering, 94*, 163-168.

666 Landstrom, K., Alsins, J., & Bergenstahl, B. (2000). Competitive protein adsorption between
667 bovine serum albumin and β-lactoglobulin during spray-drying. *Food Hydrocolloids, 14*, 75-
668 82.

669 Liu, F., Ma, C., McClements, D. J., & Gao, Y. (2016). Development of polyphenol-protein-
670 polysaccharide ternary complexes as emulsifiers for nutraceutical emulsions: Impact on
671 formation, stability, and bioaccessibility of β-carotene emulsions. *Food Hydrocolloids, 61*,
672 578-588.

673 Liu, J., Ru, Q., & Ding, Y. (2012). Glycation a promising method for food protein
674 modification. Physicochemical properties and structure, a review. *Food Research*
675 *International, 49*, 170-183.

676 Mackie, A. R., Gunning, A. P., Wilde, P. J., & Morris, V. J. (1999). Orogenic displacement
677 of protein from the air/water interface by competitive adsorption. *Journal of Colloid and*
678 *Interface Science, 210*, 157-166.

679 McCarthy, N. A., Gee, V. L., Hickey, D. K., Kelly, A. L., O'Mahony, J. A., & Fenelon, M.
680 A. (2013). Effect of protein content on the physical stability and microstructure of a model
681 infant formula. *International Dairy Journal, 29*, 53-59.

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: Springer Science+Business Media.

Millqvist-Fureby, A., Elofsson, U., & Bergenståhl, B. (2001). Surface composition of spray-dried milk protein-stabilised emulsions in relation to pre-heat treatment of proteins. *Colloids and Surfaces B: Biointerfaces, 21*, 47-58002E

Mulcahy, E. M., Mulvihill, D. M., & O'Mahony, J. A. (2016). Physicochemical properties of whey protein conjugated with starch hydrolysis products of different dextrose equivalent values. *International Dairy Journal, 53*, 20-28.

Munoz-Ibanez, M., Nuzzo, M., Turchiuli, C., Bergenståhl, B., Dumoulin, E., & Millqvist-Fureby, A. (2016). The microstructure and component distribution in spray-dried emulsion particles. *Food Structure, 8*, 16-24.

Nijdam, J. J., & Langrish, T. A. G. (2006). The effect of surface composition on the functional properties of milk powders. Journal of *Food Engineering, 77*, 919-925.

Oliver, C. M., Melton, L. D., & Stanley, R. A. (2006). Creating proteins with novel functionality via the Maillard reaction: a review. *Critical Reviews in Food Science and Nutrition, 46*, 337-50.

O'Mahony, J. A., Drapala, K. P., Mulcahy, E. M., & Mulvihill, D. M. (2017). Controlled glycation of milk proteins and peptides: functional properties. Invited review. *International Dairy Journal*, accepted manuscript.

O'Regan, J., & Mulvihill, D. M. (2010a). Heat stability and freeze–thaw stability of oil-in-water emulsions stabilised by sodium caseinate–maltodextrin conjugates. *Food Chemistry, 119*, 182-190.

O'Regan, J., & Mulvihill, D. M. (2010b). Sodium caseinate-maltodextrin conjugate stabilized double emulsions: Encapsulation and stability. *Food Research International, 43*, 224-231.

Panyam, D., & Kilara, A. (1996). Enhancing the functionality of food proteins by enzymatic modification. *Trends in Food Science & Technology, 7*, 120-125.

Paterson, A. H., Zuo, J. Y., Bronlund, J. E., & Chatterjee, R. (2007). Stickiness curves of high fat dairy powders using the particle gun. *International Dairy Journal, 17*, 998-1005.

Pisecky, J. (2012). Achieving product properties. In V. Westergaard, & E. Refstrup (Eds.), *Handbook of milk powder manufacture* (pp.163-196). GEA Process Engineering A/S.

Rosenberg, M., & Young, S. L. (1993). Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat - structure evaluation. *Journal of Dairy Science, 12*, 31–43.

Sadek, C., Pauchard, L., Schuck, P., Fallourd, Y., Pradeau, N., Le Floch-Fouéré, C., & Jeantet, R. (2015). Mechanical properties of milk protein skin layers after drying: Understanding the mechanisms of particle formation from whey protein isolate and native phosphocaseinate. *Food Hydrocolloids, 48*, 8-16.

Sadek, C., Schuck, P., Fallourd, Y., Pradeau, N., Jeantet, R., & Le Floch-Fouéré, C. (2016). Buckling and collapse during drying of a single aqueous dispersion of casein micelle droplet. *Food Hydrocolloids, 52*, 161-166.

Scholfield, C. R. (1981). Composition of Soybean Lecithin. *Journal of the American Oil Chemists' Society, 58*, 889-892.

Semenova, B. M. G., Myasoedova, M. S., & Antipova, A. S. (2001). Effect of Starch Components and Derivatives on the Surface Behaviour of a Mixture of Protein and Small-Molecule Surfactants. In E. Dickinson & M. R. (Eds.), *Food Colloids: Fundamentals of Formulation* (pp. 233–241). Cambridge: The Royal Society of Chemistry.

Sharma, A., Jana, A. H., & Chavan, R. S. (2012). Functionality of Milk Powders and Milk Based Powders for End Use Applications - A Review. *Comprehensive Reviews in Food Science and Food Safety, 11*, 518–528.

Sheu, T., & Rosenberg, M. (1998). Microstructure of microcapsules consisting of whey proteins and carbohydrates. *Journal of Food Science, 63*, 491–494.

Taneja, A., Ye, A., Jones, J. R., Archer, R., & Singh, H. (2013). Behaviour of oil droplets during spray drying of milk-protein-stabilised oil-in-water emulsions. *International Dairy Journal, 28*, 15-23.

Van Aken, G. A. (2003). Competitive adsorption of protein and surfactants in highly concentrated emulsions: Effect on coalescence mechanisms. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 213*, 209-219.

740 Vega, C., & Roos, Y. H. (2006). Invited review: spray-dried dairy and dairy-like emulsions--
741 compositional considerations. *Journal of Dairy Science, 89*, 383-401.

742 Vignolles, M.-L., Jeantet, R., Lopez, C., & Schuck, P. (2007). Free fat, surface fat and dairy
743 powders: interactions between process and product. A review. *Le Lait, 87*, 187-236.

744 Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). Milk properties. In *Dairy Science and*
745 *Technology*. 2nd Edition (pp. 159-174). CRC Press, Boca Raton, FL, USA

746 Walton, D. E. E., & Mumford, C. J. J. (1999). The Morphology of Spray-Dried Particles.
747 *Chemical Engineering Research and Design, 77*, 442-460.

748 Wilde, P., Mackie, A., Husband, F., Gunning, P., & Morris, V. (2004). Proteins and
749 emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science, 108-109*, 63-71.

750 Wooster, T. J., & Augustin, M. A. (2006). β-Lactoglobulin-dextran Maillard conjugates:
751 Their effect on interfacial thickness and emulsion stability. *Journal of Colloid and Interface*
752 *Science, 303*, 564-572.

753 Xu, D., Wang, X., Jiang, J., Yuan, F., & Gao, Y. (2012). Impact of whey protein – Beet
754 pectin conjugation on the physicochemical stability of β-carotene emulsions. *Food*
755 *Hydrocolloids, 28*, 258-266.

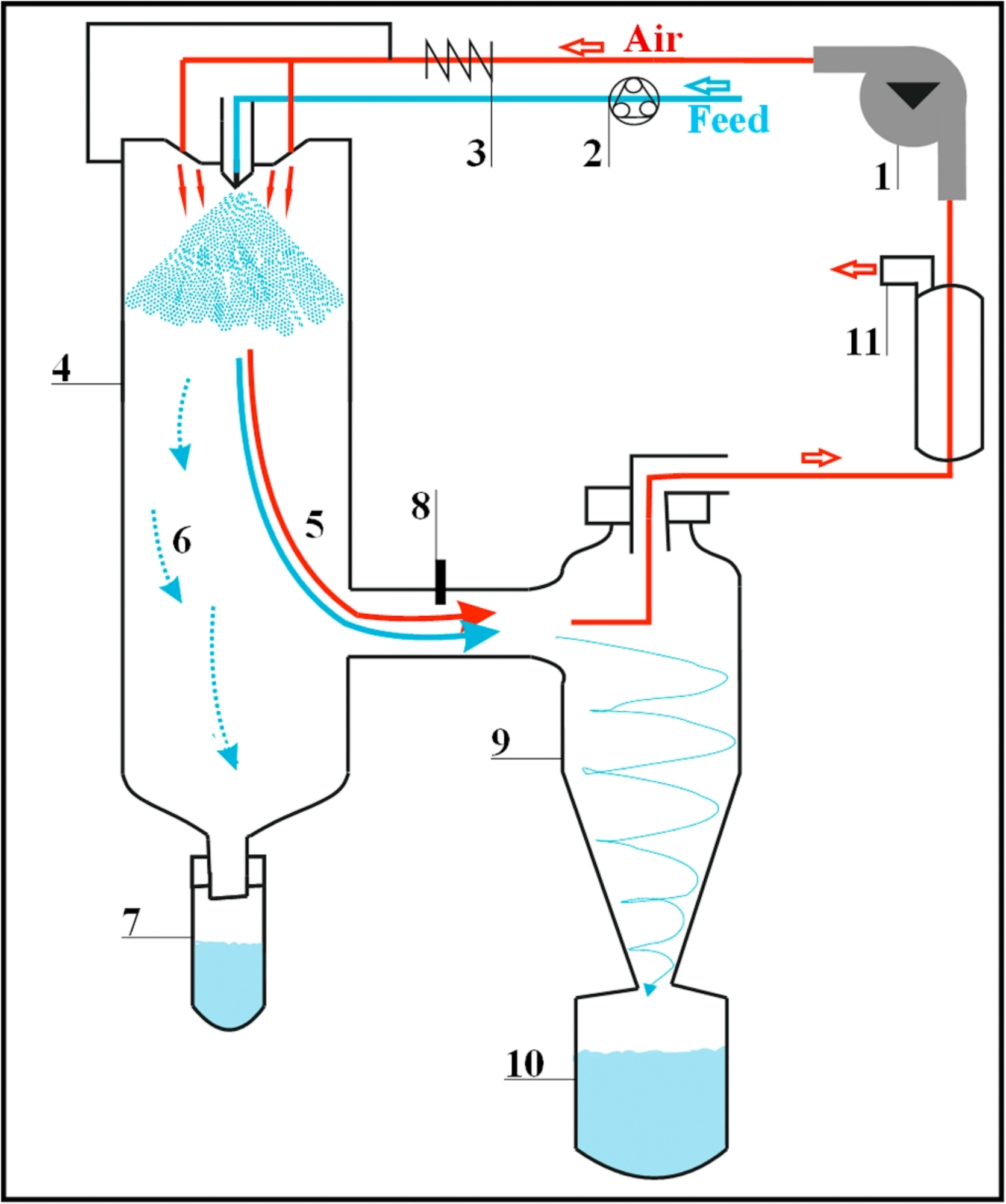
Figure captions:

Figure 1. Schematic diagram showing the set-up and the principle of operation for the laboratory-scale BÜCHI B-191 spray drier. The inlet temperature is regulated directly by the power of the heater (3) and the outlet temperature (measured at 8) is regulated indirectly by controlling the feed flow rate (2) and the air flow (1). Feed is introduced into the main drying chamber (4) by a 2-fluid nozzle atomiser, where it is rapidly dried by heated air; dried particles are pulled into the cyclone (9) by the means of an aspirator (1). Large and heavy particles (i.e., wet lumps and scorched particles, falling off the build-up around the nozzle and around hot air inlets, respectively) are separated from the powder by means of the air pull and gravity (5 and 6, respectively). By design, air pull is insufficient to move larger and heavier particles into the cyclone, making them fall into the waste collection container (7) at the bottom of the dryer main chamber. Dried powder particles are further separated from fines in the cyclone and the final powder is collected in the powder collection container (10) at the bottom of the cyclone. The clarified air is exhausted at the top of bag filter (11).

Figure 2. Differences in the build-up of fine powder on the wall of the cyclone during spray-drying of powders ($_p$) containing different emulsifier systems: whey protein isolate (WPI_p), whey protein hydrolysate (WPH_p), WPH + CITREM ($WPH+CIT_p$), WPH + lecithin ($WPH+LEC_p$) and WPH-maltodextrin conjugate ($WPH-MD_p$). The powders were produced using a laboratory-scale spray dryer (BÜCHI B-191). The photographs were taken ~30 min after starting the drying run for all powders.

Figure 3. Particle size distribution for (A) homogenised emulsions (dryer feeds), model infant formula powders (B) after spray-drying and (C) after powder reconstitution. The formulations contained different emulsifier systems: (×) whey protein isolate, (□) whey protein hydrolysate, (▲) WPH + CITREM, (●) WPH + lecithin and (−) WPH-maltodextrin conjugate. The powders were produced using a laboratory-scale spray dryer (BÜCHI B-191).

Figure 4. Scanning electron microscope (SEM; A and B) and confocal laser scanning microscope (CLSM; C) images of model infant formula powders ($_p$) containing different emulsifier systems: whey protein isolate (WPI_p), whey protein hydrolysate (WPH_p), WPH + CITREM ($WPH+CIT_p$), WPH + lecithin ($WPH+LEC_p$) and WPH-maltodextrin conjugate ($WPH-MD_p$). For the CLSM analysis powders were labelled with Nile Red:Fast Green (3:1) and the micrographs show distribution of oil droplets (green) and protein particles (red). Scale bar for the CLSM micrographs = 5 μm. The powders were produced using a laboratory scale spray dryer (BÜCHI B-191).


Air
Feed
1
2
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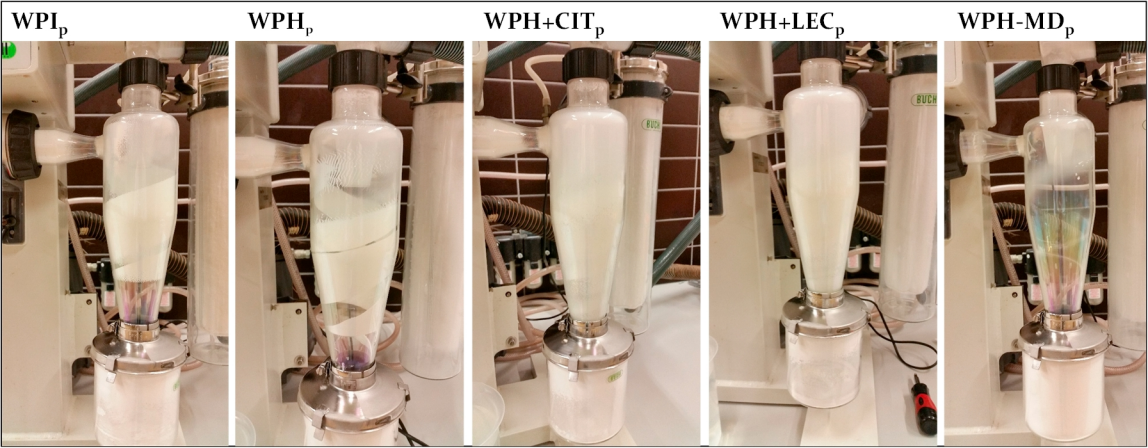
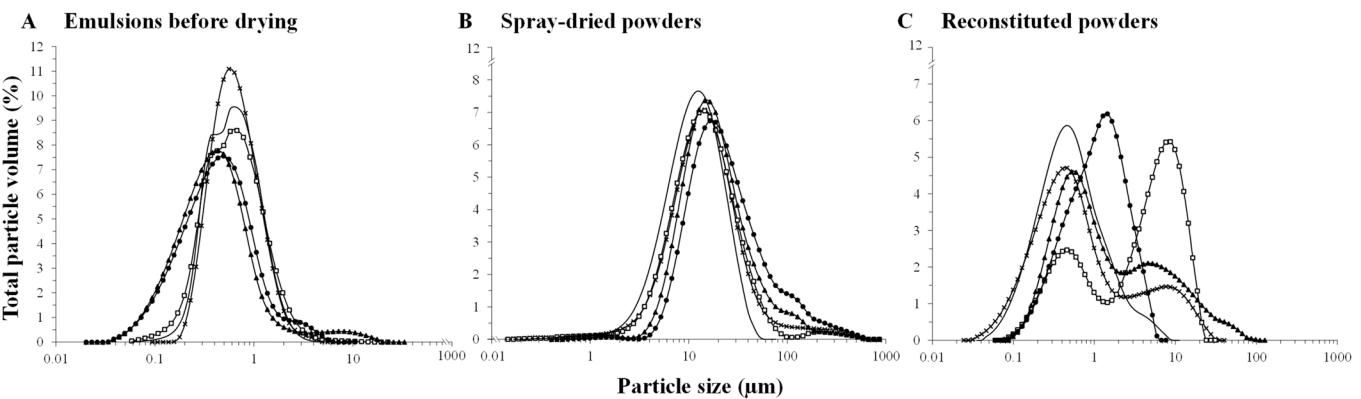
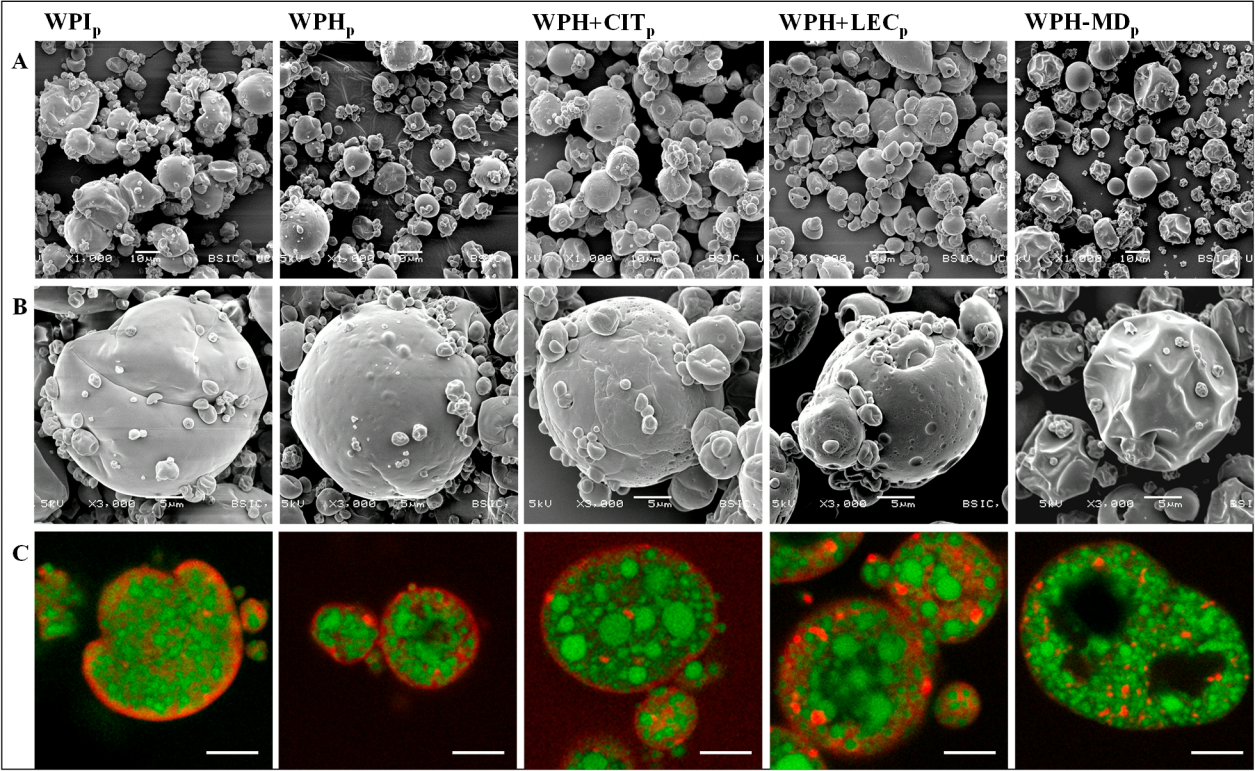

WPI$_p$
WPH$_p$
WPH+CIT$_p$
WPH+LEC$_p$
WPH-MD$_p$


A
Emulsions before drying
B
Spray-dried powders
C
Reconstituted powders
Total particle volume (%)
Particle size (µm)
0.01
0.1
1
10
100
1000


WPI$_p$
WPH$_p$
WPH+CIT$_p$
WPH+LEC$_p$
WPH-MD$_p$
A
B
C

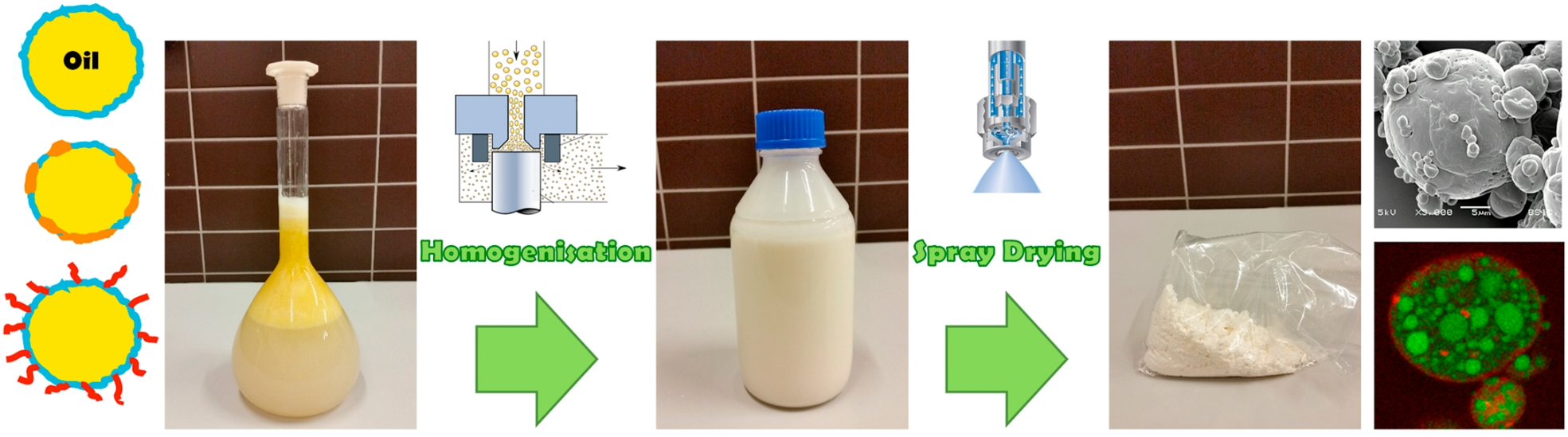

Oil
Homogenisation
Spray Drying
Emulsifiers
Protein
Lipid
Conjugate
→ Emulsion Stability
→ Processing Performance
→ Powder Properties

Table 1. Characteristics of emulsions prepared using different emulsifiers; whey protein isolate (WPI_e), whey protein hydrolysate (WPH_e), WPH + CITREM ($WPH+CIT_e$), WPH + lecithin ($WPH+LEC_e$) and WPH-maltodextrin conjugate ($WPH-MD_e$), used to produce model infant formula powders.

		Emulsions				
Emulsion characteristics		**WPI_e**	**WPH_e**	**$WPH+CIT_e$**	**$WPH+LEC_e$**	**$WPH-MD_e$**
Total solids content	(%, w/w)	32.6 ± 0.16[a]	32.2 ± 0.69[a]	32.5 ± 0.10[a]	32.2 ± 0.04[a]	32.7 ± 0.18[a]
PSD[1] (µm)	**$D_{4,3}$**	0.76 ± 0.05[a]	0.78 ± 0.14[a]	0.81 ± 0.21[a]	0.58 ± 0.06[a]	0.67 ± 0.05[a]
	$D_{v,0.1}$	0.25 ± 0.07[a]	0.21 ± 0.04[a]	0.11 ± 0.07[a]	0.15 ± 0.01[a]	0.24 ± 0.05[a]
	$D_{v,0.5}$	0.55 ± 0.06[a]	0.55 ± 0.01[a]	0.38 ± 0.08[a]	0.46 ± 0.12[a]	0.55 ± 0.03[a]
	$D_{v,0.9}$	1.26 ± 0.10[a]	1.40 ± 0.12[a]	1.07 ± 0.07[a]	1.52 ± 0.85[a]	1.23 ± 0.04[a]
Flow behaviour[2]	**η_{300}** (mPa.s)	13.5 ± 0.55[a]	11.9 ± 1.27[ab]	13.0 ± 0.49[a]	11.9 ± 0.24[ab]	10.9 ± 0.31[b]
	K (Pa.s^n; x10^2)	1.57 ± 0.19[a]	1.18 ± 0.22[a]	2.92 ± 0.87[a]	1.64 ± 1.25[a]	2.19 ± 0.50[a]
	n	0.97 ± 0.02[a]	1.00 ± 0.02[a]	0.85 ± 0.06[a]	0.98 ± 0.16[a]	0.87 ± 0.05[a]

[1] Particle size distribution parameters: $D_{4,3}$, volume mean diameter of oil globules; $D_{v,0.1}$, $D_{v,0.5}$, and $D_{v,0.9}$ representing particle size in the 10%, 50% and 90% quantiles of the distribution.

[2] Flow behaviour parameters; (η_{300}) apparent viscosity measured at 300 s^{-1}; (K) consistency coefficient; (n) flow behaviour index.

[(a-b)] Values for a given parameter (i.e., within each row) for all powders, not sharing a common superscript differed significantly ($P < 0.05$).

Table 2. Composition and colour of model infant formula powders ($_p$) produced with different emulsifier systems: whey protein isolate (WPI_p), whey protein hydrolysate (WPH_p), WPH + CITREM ($WPH+CIT_p$), WPH + lecithin ($WPH+LEC_p$) and WPH-maltodextrin conjugate ($WPH-MD_p$). The powders were produced using a laboratory-scale spray dryer (BÜCHI B-191).

Powder	Composition (%, w/w)					Colour coordinates		
	Protein	Fat	Carbohydrate	Ash	Moisture	L*	a*	b*
WPI_p	12.1 ± 0.21^{a}	28.4 ± 1.33^{a}	57.7 ± 0.99^{a}	0.52 ± 0.17^{a}	1.73 ± 0.35^{a}	96.1 ± 0.26^{a}	-1.26 ± 0.09^{b}	3.15 ± 0.24^{a}
WPH_p	12.6 ± 0.10^{b}	29.0 ± 1.58^{a}	56.1 ± 1.50^{a}	0.67 ± 0.10^{ab}	1.08 ± 0.66^{a}	96.3 ± 0.16^{a}	-1.30 ± 0.11^{b}	3.02 ± 0.15^{a}
$WPH+CIT_p$	12.3 ± 0.13^{ab}	28.8 ± 0.34^{a}	56.6 ± 0.43^{a}	0.87 ± 0.19^{ab}	1.36 ± 0.91^{a}	95.8 ± 0.49^{ab}	-1.26 ± 0.06^{b}	3.35 ± 0.26^{a}
$WPH+LEC_p$	12.7 ± 0.22^{b}	26.9 ± 2.44^{a}	58.2 ± 1.84^{a}	0.71 ± 0.13^{ab}	1.48 ± 0.34^{a}	93.8 ± 1.28^{c}	-1.96 ± 0.08^{a}	6.37 ± 0.25^{c}
$WPH-MD_p$	12.5 ± 0.09^{b}	26.9 ± 2.56^{a}	58.8 ± 3.17^{a}	0.97 ± 0.13^{b}	0.89 ± 0.34^{a}	94.1 ± 0.52^{bc}	-0.85 ± 0.07^{c}	4.77 ± 0.38^{b}

$^{(a-c)}$ Values for a given parameter (i.e., within each column) for all powders, not sharing a common superscript differed significantly ($P < 0.05$).

Table 3. Properties of spray dried model infant formula powders ($_p$) prepared with different emulsifier systems: whey protein isolate (WPI_p), whey protein hydrolysate (WPH_p), WPH + CITREM ($WPH+CIT_p$), WPH + lecithin ($WPH+LEC_p$) and WPH-maltodextrin conjugate ($WPH-MD_p$). The powders were produced using a laboratory-scale spray dryer (BÜCHI B-191).

Powder characteristics		WPI_p	WPH_p	$WPH+CIT_p$	$WPH+LEC_p$	$WPH-MD_p$
Drying performance[1]	**Powder recovery (%)**	22.0 ± 6.59^{a}	26.1 ± 3.27^{a}	21.3 ± 6.67^{a}	18.1 ± 2.56^{a}	55.3 ± 10.8^{b}
	Stickiness (relative)	+	+	++	++	-
PSD (µm) Powders[2]	**$D_{4,3}$**	26.5 ± 16.9^{ab}	25.4 ± 4.79^{ab}	30.8 ± 2.94^{ab}	41.1 ± 13.2^{a}	14.2 ± 4.79^{b}
	$D_{v,0.1}$	5.75 ± 0.56^{a}	5.85 ± 0.21^{a}	7.87 ± 0.54^{b}	9.52 ± 0.73^{c}	4.76 ± 0.27^{a}
	$D_{v,0.5}$	15.5 ± 2.29^{ab}	15.1 ± 0.33^{ab}	18.4 ± 1.64^{bc}	22.7 ± 2.41^{c}	12.2 ± 0.94^{a}
	$D_{v,0.9}$	59.5 ± 48.3^{a}	40.4 ± 3.22^{a}	56.0 ± 15.4^{a}	95.1 ± 43.6^{a}	26.6 ± 2.33^{a}
	% <5 µm	10.5 ± 2.16^{bc}	13.5 ± 0.71^{b}	6.33 ± 1.64^{cd}	2.84 ± 0.81^{d}	19.9 ± 2.71^{a}
	% >100 µm	2.93 ± 6.92^{a}	2.26 ± 1.13^{a}	4.05 ± 0.93^{a}	7.78 ± 5.29^{a}	0.00 ± 0.00^{a}
Contact angle (θ)		42.1 ± 0.08^{b}	36.9 ± 1.45^{d}	46.7 ± 1.00^{a}	40.5 ± 2.27^{bc}	37.2 ± 0.91^{cd}
Surface free fat (%)		14.1 ± 2.68^{a}	22.9 ± 4.85^{a}	20.0 ± 5.05^{a}	25.4 ± 17.9^{a}	13.3 ± 1.18^{a}
Surface composition (%)	**Protein**	50.7 ± 6.42^{a}	37.1 ± 6.22^{b}	27.0 ± 2.81^{b}	29.1 ± 4.03^{b}	32.3 ± 2.02^{b}
	Fat	34.1 ± 9.42^{a}	50.9 ± 6.47^{ab}	64.2 ± 6.22^{b}	61.8 ± 6.82^{b}	50.0 ± 3.23^{ab}
	Carbohydrate	15.2 ± 3.02^{ab}	12.0 ± 0.91^{ab}	8.85 ± 3.50^{b}	9.12 ± 3.17^{b}	17.7 ± 1.61^{a}
PSD (µm) Reconstituted[2]	**$D_{4,3}$**	2.42	5.72	5.00	1.47	0.84
	$D_{v,0.1}$	0.15	0.35	0.31	0.35	0.17
	$D_{v,0.5}$	0.57	4.68	1.10	1.18	0.51
	$D_{v,0.9}$	8.02	13.3	14.4	3.07	1.82

[1] Drying performance describing powder recovery (%, w/w total solids, TS; powder TS/feed TS); stickiness classification: -, non-sticky; +, moderately sticky; ++, very sticky.

[2] Particle size distribution parameters: $D_{4,3}$, volume mean diameter; $D_{v,0.1}$, $D_{v,0.5}$, and $D_{v,0.9}$ representing particle size in the 10%, 50% and 90% quantiles of the distribution. Particle size distribution analysis for reconstituted powders was carried out only on one trial.

[(a-d)] Values for a given parameter (i.e., within each row) for all powders, not sharing a common superscript differed significantly ($P < 0.05$).