Reduction of surface fat formation on spray-dried milk powders through emulsion stabilization with λ-carrageenan

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Abstract

The appearance of surface fat during the atomization process in spray drying of milk particles often impairs the functional powder properties. To investigate a possible approach that could minimise the surface fat formation, the interaction between a whole milk model emulsion and λ-carrageenan at various concentrations was studied, as well as how it influences the atomization behaviour and the resulting particle characteristics. Carrageenan can stabilize emulsions in the presence of milk protein by adsorption on the milk fat globule membranes. If too little or too much of the polysaccharide was added, bridging flocculation or depletion flocculation, respectively, occurred inside the emulsions. The best stability and minimal fat globule size were obtained for a carrageenan content of 0.3% w/w. Rheological investigation indicated that the extensional viscosity can be an important factor influencing the emulsion disintegration behaviour during atomization. The λ-carrageenan stabilized emulsions featured a significantly increased extensional viscosity and a better fat encapsulation in the corresponding spray-dried particles, promoting solubility and oxidative stability. Surface fat extraction showed that the most stable emulsion lead to particles with the least amount of surface fat. Though the surface of these particles was still covered by fat according to spectroscopic analysis, this surface fat layer was very thin in comparison to carrageenan-free powder as observed by confocal microscopy. Yet, the addition of carrageenan was also found to have one adverse effect on the intended powder properties, as the strengthened emulsion network translated into denser particles and thus a deterioration of the powder’s reconstitution behaviour.

Keywords: Milk powder; Carrageenan; Spray drying; Surface fat; Atomization; Emulsion stabilization

1 Introduction

Whole milk, skim milk and infant formula emulsions are spray-dried to powder form at large industrial scale in order to accomplish better preservation, reduced bulk volume for economy of transportation and easier processing as food ingredients. However, during spray drying usually an unwanted layer of fat occurs on the particles’ surface and this leads to detrimental effects on the powder properties, including reduced solubility in water (Fäldt & Bergenstål, 1996; Miljövård, Fureby, Elofsson, & Bergenståhl, 2001), faster expiration due to lipid oxidation (Granelli, Fäldt, Appelqvist, & Bergenståhl, 1996; Hardas, Danviriyakul, Foley, Nawar, & Chinachoti, 2000; Keogh et al., 2001) and greater stickiness (Kim, Chen, & Pearce, 2005a; Nijdam & Langrish, 2006). This can mean a deteriorated product quality for the end user and a reduction in efficiency during manufacturing due to significant product loss and the necessity of additional processing steps, such as lecithination. For this reason, it is important to identify the driving forces that cause the formation of surface fat during spray drying of milk powder. A few studies previously speculated that the actual drying stage of the spray drying process might not be primarily responsible, but rather an atomization induced mechanism would already determine the eventual chemical surface composition (Fyfe, Kravchuk, Nguyen,
The hypothesis was supported by recent studies differentiating the impact of the atomization stage from the drying stage (Foerster, Gengenbach, Woo, & Selomulya, 2016a; Wu et al., 2014). By comparing the surface composition of the spray-dried particles with the atomized droplets, which were cryogenically flash-frozen immediately after leaving the nozzle, it was learnt that the freshly generated droplets were already covered by a fat film and that this surface fat coverage remained relatively unchanged throughout the following drying process. It was concluded that the surface fat content is hence not significantly reducible by modifying the spray drying conditions. Also, for the range of atomization nozzles investigated, the atomization triggered fat accumulation on the droplet surfaces was independent of the atomization technique. Instead, a promising way to reduce the amount of surface fat seems to modify the emulsion prior to spray drying in order to moderate the segregation between the lipid and the aqueous phase during atomization. Further, it is crucial to understand the actual mechanism that causes the emulsions to disintegrate in a way that results in the fat being present at the surface as soon as individual droplets are formed.

Therefore, the objectives of the present study were, firstly, to contribute towards a better insight into this mechanism and, secondly, to investigate a potential method to improve the properties of milk powder by modifying the emulsion to be spray-dried. Towards the first aim, milk model emulsions of different compositions were investigated in terms of their break-up behaviour under sheared and extensional stress. There are various possible approaches to address the second aim. As studies have shown, adding a surfactant, such as polysorbate 80 or lecithin, to milk emulsions or oil/milk protein emulsions and subsequent co-spray drying enhanced the product powders' wettability (Fonseca, Bento, Quintero, Gabas, & Oliveira, 2011; Lalibeeharry et al., 2014; Millqvist-Fureby & Smith, 2007). Yet, it was also argued that successive spray drying and lecithin coating, as typically done on industrial scale, still remains more efficient (Tian et al., 2014). Instead, milk emulsion could be modified in a way that strengthens their stability in order to bring about a greater fat encapsulation upon spray drying, thus making subsequent coating redundant.

Firstly, by emulsion stability analyses of milk protein containing oil/water emulsions, heat treatment at temperatures above 60 °C (Millqvist-Fureby et al., 2001), a pH value reduction (Dalgleish, 1997) and addition of calcium ions (Agbola & Dalgleish, 1996a, 1996b) were all shown to be not successful in this regard. However, through thermal pre-treatment at 80 °C Wang, Liu, Chen, and Selomulya (2016) attained a cross-linked emulsion of whey protein isolate and fish oil, whose corresponding spray-dried powders featured improved inhibition of lipid oxidation. Secondly, the fat globule size inside oil/water emulsions also influences the emulsion stability and thus the amount of surface fat, for instance by mechanical treatment during homogenization via microfluidization or ultrasonication. Yet, the ideal size depends on the type of oil, homogenization technique and spray-dried particle diameter (Jafari, Assadpour, Bhandari, & He, 2008; Munoz-Ibanez et al., 2016; Soottitantawat, Yoshii, Furuta, Okawara, & Linko, 2003). Thirdly, it has been demonstrated that the adsorption of certain polysaccharides on the membranes around the fat globules of various emulsions can enhance the stability against environmental influences, such as thermal and mechanical stress (Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007; Gharsallahou et al., 2010; Guzey, Kim, & McClements, 2004). A prominent example is pectin, which was for instance found to improve the storage stability of sunflower oil emulsion with whey protein isolate and sodium caseinate as emulsifiers (Einhorn-Stoll, Ulbrich, Sever, & Kunzek, 2005). Serfert et al. (2013) obtained enhanced fish oil encapsulation and oxidative stability of powders from an emulsion that comprised of glucose syrup, whey protein and pectin at pH 4. However, while this polysaccharide with its negatively charged carboxylate groups can adsorb at casein below the latter's isoelectric point (pH of approximately 4.6) and is thus used in stabilization of acid dairy drinks, it is not effective at the pH of milk itself (6.4–6.7) (Kravtchenko, Parker, & Trespoej, 1995; Suru, Decker, & McClements, 2006). In contrast, carrageenan, a linear sulphated polysaccharide, also undergoes attractive electrostatic interaction with κ-casein at neutral milieu (Dalgleish & Morris, 1988; Dickinson, 1998) and therefore is used as stabilizer in pH neutral dairy beverages that are commercially available (Bixler, Johndro, & Falshaw, 2001; FAO, 1987; Yanes, Durán, & Costell, 2002). The European Food Safety Authority and the US Food & Drug Association, for instance, have concluded that carrageenan as a food additive is considered as safe (FDA, 2016; SCF, 2003).

Carrageenan is a natural hydrocolloid extracted from red algae and is widely used in food applications to form gels and stabilize beverages. It features one, two or three sulphate groups per disaccharide in its kappa (κ), iota (ι) and lambda (λ) form, respectively. Singh, Tanehama, Hemar, and Munro (2003) observed an improvement in creaming stability of a soya oil emulsion that contained 3% w/w sodium caseinate with increasing κ-carrageenan content from 0 to 0.4% w/w. For a caseinate content of 0.5% w/w, however, addition of κ-carrageenan impaired the stability due to flocculation. An emulsion containing flaxseed oil and whey protein reached substantially better stability when mixed with any of the three main carrageenan types, with the best result for λ-carrageenan (Stone & Nickerson, 2012). In skim milk emulsions, ι- and κ-carrageenan were found to induce depletion flocculation at temperatures above their coil-helix transition temperature (which is below 60 °C), because only the charge density of their helix structure was great enough to allow adsorption at the casein membranes (Langendorf, Cuvelier, Michon, Launay, & Parker, 2000). Yet, λ-carrageenan remains an active stabilizer at elevated temperature, which might be particularly beneficial for spray-drying applications. Furthermore, the higher density of anionic sulphate groups in the λ-form brings along the advantage of gel inhibition and good water solubility even at lower temperature (room temperature).

A few reports have been published about the stabilization of oil/water emulsions in the presence of carrageenan and one type of milk protein. However, there is a lack of investigation hitherto on the impact of carrageenan for a more complete model system that is representative of whole milk, comprising of all its main components (lactose, whey protein, casein and fat). In addition, carrageenan’s subsequent effect on the atomization behaviour during spray drying and thus the powder properties, particularly in respect to surface fat formation, is still to be studied. In the first part of the present study, a milk model emulsion was investigated to find the optimum λ-carrageenan content in terms of fat globule size and emulsion stability. In the second part, it was studied whether the stabilization with λ-carrageenan translated into optimized powder properties after spray drying. For this purpose, the powders (and selected atomized emulsion droplets) were analysed in respect to their component distribution via X-ray photoelectron spectroscopy (XPS), confocal laser scanning microscopy (CLSM) and surface fat extraction. Powder properties including morphology, porosity, solubility, wettability and oxidative stability were compared for different carrageenan concentrations. An important connecting link between modified emulsions and spray-dried particle properties was the emulsions’ behaviour during atomization. Shear and extensional viscosity analyses gave new insights into the disintegration process and how it is influenced by fat and hydrocolloid content.
2 Material and methods

2.1 Emulsion preparation

A milk model emulsion, which resembled the composition of bovine whole milk, was stabilized with λ-carrageenan at various concentrations. The emulsion contained 40.8% w/w lactose, 31.1% w/w fat and 27.0% w/w protein in dry matter (d.m.) and featured a solid content of 20% w/w. It was prepared by dissolving lecithin free, commercial skim milk powder (home brand from Coles Supermarkets Australia Pty Ltd, Australia) in Milli-Q purified water (Merck KGaA, Germany) with stirring at 47 °C for 1 h. Subsequently, sustainably sourced refined *Elaisis guineensis* palm fruit oil (Aurora Pty Ltd, Australia), with a slip melting point of 37-39 °C and a free fatty acid content (as palmitic) of less than 0.09%, was added. Following prehomogenization in a high-speed colloid mill (WiseMix Homogenizer HG-15D, Daihan Scientific, South Korea) at 1000 rpm for 1 min, different amounts of λ-carrageenan (Melbourne Food Depot Pty Ltd, Australia) were added to form carrageenan contents of 0, 0.1, 0.2, 0.3, 0.4 and 0.5% w/w in respect to the total emulsion mass. The emulsions were stirred for further 30 min at 47 °C and then were homogenized in a high pressure homogenizer (EmulsiFlex-C5, Avestin, Canada) with three passes at 1350 bar and two subsequent passes at 650 bar. The homogenization temperature was not directly controlled, but the sample temperature was measured to be between 45 °C at the beginning of the homogenization process and 35 °C at the end. The emulsions were stored at room temperature and were spray-dried and analysed by the following procedures within 3 h after preparation if not stated otherwise.

2.2 Emulsion analysis

2.2.1 Turbiscan emulsion stability, emulsion microstructure and pH value

24 h instability tests of each emulsion were conducted with a Turbiscan Classic MA2000 multiple light scattering instrument (Formulation SA, France) with a pulsed near infrared light source (wavelength of 850 nm). The backscattering fraction as a function of the emulsion height inside a cylindrical glass measurement cell was determined every 30 min. These results were compared with the microstructure of original emulsion samples that were stored at 6 °C for up to ten days. Images were taken at various locations inside emulsion drops that were contained between a glass microscope slide and a cover slip under 10× magnification with a conventional optical microscope (B1-211A, Motic, P.R. China). The pH value was measured with a benchtop pH meter (H4212, Hanna Instruments, USA) after homogenization.

2.2.2 Fat globule size distribution inside original emulsions and after atomization process

The emulsion droplet size, henceforth described as fat globule size in order to distinguish from the droplet size of the sprays after atomization in the spray dryer nozzle, was analysed by laser light scattering using a Mastersizer 2000 apparatus (Malvern Instruments, UK) equipped with a Hydro 2000G wet cell. Approximately 250 ml of the pre-diluted emulsions (oil content of 0.01% w/w) were added to the wet cell, which was filled with about 1 l of Milli-Q purified water, to establish a laser obscuration level of 5.2-5.5% to avoid multiple scattering effects. The instrument operated at laser wavelengths of 633 and 466 nm. The pump and stirrer speeds were set to 1000 rpm and 500 rpm, respectively. The physical properties of the fat globules were described with a refractive index of 1.462 and an absorbance index of 0.1. Four samples were taken from each emulsion to conduct individual measurements of 20 s duration, the standard deviation was determined from this and the average was reported as the mean diameter over volume:

\[
D(43) = \frac{\sum \left( n_d d_i^3 \right)}{\sum \left( n_d^2 \right)}
\]

where the particle diameter and number of particles in each size class are expressed by \( d_i \) and \( n_i \), respectively. The size distribution and the corresponding volumetric mean diameters were determined for the original emulsions after homogenization. In addition, they were also measured for the same emulsions after they had been passed through a spray drying atomization nozzle. The nozzle was operated outside of the spray dryer and the atomized emulsions were collected immediately after leaving the nozzle, and thus no contact with drying air occurred. Two different atomization techniques were investigated: a pressure-swirl single-fluid spray nozzle (MT Brass Series, AmFog Nozzle Technologies Inc, USA) with an orifice of 0.2 mm in diameter at a feed pressure of 500 kPa, and a microfluidic jet nozzle that is described in detail in section 2.3.1.

2.2.3 Shear viscosity and extensional viscosity of the emulsions

Dynamic shear viscosities were measured at 25 °C with a cone and plate rheometer (Haake Mars, Thermo Fisher Scientific, USA), which was equipped with a MP60 measuring plate and a C60/1 cone rotor. Each sample's viscosities were investigated at six different shear rates in the range of 50-500 1/s, and the average of three samples taken from the same emulsion was determined together with the respective standard deviation. The extensional viscosity was of special interest for interpretation of the jet disintegration mechanism of the microfluidic jet nozzle described in section 2.3.1. For a fluid that does not follow Newtonian behaviour, such as the emulsions investigated in this study (see Fig. 3b), no assumption can be made about the relationship between its viscosity under shear stress and its viscosity under extensional stress. The viscosity of the milk model emulsion was too low for standard capillary-breakup extensional rheometry, however. Therefore, acoustically-driven microfluidic extensional rheometry was employed, which is designed to capture the extensional viscosity of low-viscosity samples (McDonnell, Gopesh, et al., 2015; McDonnell, Jason, et al., 2015). These measurements were conducted at room temperature (25 °C) at the School of Engineering, RMIT University in Melbourne, Australia. The rheometer and the mathematical analysis procedure have been described elsewhere (Bhattacharjee, McDonnell,
Prabhakar, Yeo, & Friend, 2011). In brief, a sessile droplet of 1 μl in volume (i.e. approximately 1.2 mm in diameter) was placed onto the piezoelectric substrate surface of a surface acoustic wave (SAW) device. A pulse of SAW energy was then applied to the droplet, causing it to elongate and to form a stable liquid bridge between the substrate and a parallel opposing surface at a gap length of 1.5 mm. At this point the SAW pulse was terminated, allowing the liquid bridge to thin under capillary forces and thus creating a uniaxial extensional flow. The diameter \( \rho \) at the neck of the thinning emulsion filament was recorded with a high-speed camera until it had decreased to half of its initial value \( (D_0) \) (see Fig. 1a as well as Video 1, which is available in the online version of the supplementary information). The evolution of the filament diameter with respect to time was determined with standard image analysis techniques. From this, the Ohnesorge number \( Oh \) could be determined, allowing calculation of the extensional viscosity \( \eta_{ext} \) by the following expression:

\[
\eta_{ext} = 3 \cdot Oh \cdot \sqrt{\rho \cdot \gamma \cdot D_0 / \rho} \tag{2}
\]

where \( \rho \) was the emulsion density and \( \gamma \) the initial surface tension of the emulsion at 25 °C as taken from Bertsch (1983). Every sample was analysed by measurement of three individual droplets with ten runs per droplet, and from these the average value was taken and the standard deviation was calculated. As the intention of this analysis was to study the impact of the fat globules on the film disintegration, the emulsion preparation was slightly modified for the purpose of the extensional viscosity measurements. A mixture of lactose, calcium caseinate and whey protein powder was used instead of skim milk powder. Otherwise it would not have been possible to prepare emulsions with very low fat content, as skim milk powder itself still contains a significant amount of fat. Thus, a low-fat emulsion that contained hardly any fat (0.3% w/w in d.m.) could be compared with regular-fat emulsions featuring 31.1% w/w fat in d.m. (and 0 or 0.3% w/w carrageenan). All of these emulsions had a solid content of 20% w/w, and their ratio of lactose to protein as well as their proportion between caseinate and whey protein were equivalent to the earlier described standard emulsions.
2.3 Powder analysis

2.3.1 Powder production with microfluidic jet spray dryer

A microfluidic jet spray dryer, comprehensively described elsewhere (Foerster et al., 2016a), was used to dry the emulsions under production of monodisperse particles with uniform drying history. In short, the utilized atomization nozzle consisted of a glass tube with an orifice of 100 μm in diameter. A piezoelectric ceramic jacket surrounded the nozzle tip to impose a sinusoidal pulse of 12 kHz, causing disintegration of the feed emulsion jet into individual droplets with uniform size (see Fig. 1b). The feed was pushed through the nozzle by pneumatic pressure of 34-53 kPa for respective carrageenan contents of 0-0.5% w/w to account for the increasing viscosity. The nozzle was either operated detached from the spray drying apparatus for cryogenic flash-freezing of the atomized droplets (described in section 2.3.2) or was positioned onto the drying tower for spray drying. The tower was 3.2 m in height and contained a concurrent drying air flow. The temperatures of the drying air at the top and bottom of the drying chamber were 200 °C and 88 °C, respectively.

2.3.2 Cryogenic flash-freezing of atomized droplets

To facilitate the study of the chemical surface composition and internal component distribution of emulsion droplets as present immediately after atomization, a modified methodology of Rogers et al. (2008) was applied. Milk model emulsion droplets with 0.3% w/w carrageenan content were flash-frozen in liquid nitrogen at a distance of 10 cm from the microfluidic jet nozzle, which was operated outside of the spray dryer. Afterwards, the droplets were kept in frozen state by cooling with dry ice until freeze-drying was carried out for 48 h at 0.1 mbar and with a collector temperature of −80 °C in a FreeZone 2.5 l benchtop freeze-dryer (Labconco Corp., USA). As the microfluidic atomization technique generated monodisperse particles, the mean size of the atomized droplets as well of the corresponding spray dried particles could be determined by simple image analysis of a small powder sample (100 particles) with a light microscope (B1-211A, Motic, P.R. China) under 4× magnification.

2.3.3 Surface fat extraction

A modified method of the gentle surface fat extraction method proposed by Wang et al. (2016) was applied. This approach avoided fat removal from the inner part of the particles by limiting the time exposed to the solvent medium. While the free fat on the surface of a powder particle is quickly dissolved by organic solvents, the free fat in the inner part of the particles is extracted considerably slower (Kim, Chen, & Pearce, 2005b). 1 g of powder was weighed out on a filter paper with a pore size of 2.5 μm (Grade 1803, Filtech Pty Ltd, Australia), which was subsequently transferred into a Büchner funnel. The funnel was filled with 30 ml of petroleum ether (boiling point of 40-60 °C, Sigma-Aldrich Pty Ltd, Australia), wherein the powder rested for 2 min. Afterwards, vacuum filtration was commenced and the powder was washed three times with 20 ml of petroleum ether per pass. The powder filled filter paper was then kept in a drying oven at 33 °C for 24 h for evaporation of any remaining ether residue, prior to a second weighing to determine the extracted amount of surface fat. The study was conducted immediately after spray drying and the original powder samples were also stored at 33 °C ahead of the fat extraction process to eliminate any impact of adsorbed air humidity on the mass measurements. The surface fat content was determined for three powder samples that were collected for about 20 min each during the same spray drying run at 40, 60 and 80 min after a steady column temperature profile had been reached, and the standard deviation was determined. The amount of extracted free fat was presented as percentage relative to the particles' original total fat content.

2.3.4 Spectroscopic surface composition measurements

The chemical surface composition of selected powders was estimated by XPS investigation with an AXIS Nova spectrometer (Kratos Analytical Inc., UK). The spray-dried particles of all carrageenan contents were analysed, as well as the atomized droplets with 0.3% w/w carrageenan after cryogenic flash-freezing/freeze-drying and the spray-dried powders from emulsions with 0.1 and 0.3% w/w carrageenan after surface fat extraction. The instrument was equipped with a monochromated Al Kα X-ray source and a hemispherical analyser that was operated in the fixed analyser transmission mode with the standard aperture (0.3 mm × 0.7 mm analysis area). The pressure inside the main vacuum chamber was of the order of 10−8 mbar. Shallow wells of a custom-built sample holder contained the powders, and two different locations were analysed for each sample at a nominal photoelectron emission angle of 0° with respect to the surface normal. The actual emission angle is ill-defined in the case of particles (ranging from 0° to 90°), and hence the sampling depth ranged from 0 nm to approximately 10 nm. All detected elements were identified from survey spectra. The respective relative atomic concentrations were determined from the integral peak intensities and sensitivity factors provided by the manufacturer. The concentrations in lactose, protein and fat can be interpreted as linear combination of the atomic surface composition (P Fälldt, Bergenstahl, & Carlsson, 1993). Each component's fraction at the surface, expressed in atomic concentration, was thus estimated by linearization based on the representative structural formulas of lactose, milk protein and milk fat, as elaborated by Chew et al. (2014).

2.3.5 Confocal laser scanning microscopy

The protein and fat distributions inside particles obtained from spray drying of milk model emulsions with 0, 0.3 and 0.5% w/w carrageenan content were investigated by CLSM. The microscopy technique and the preceding labelling process with
2.3.6 Scanning electron microscopy

The morphology of the spray-dried particles was imaged with a field-emission scanning electron microscope (SEM) (FEI Nova NanoSEM 450 FE-SEM, FEI Corp, USA) using a 5 kV electron beam. The internal features and porosity of particles that had been intentionally cleaved asunder with a thin razor blade were studied as well as the exterior morphology before and after surface fat extraction. The samples were coated with a 2 nm thick Iridium layer to prevent them from electrical charging.

2.3.7 Powder dissolution and wetting behaviour

Two studies to evaluate the reconstitution behaviour of the spray-dried powders were undertaken. First, focused beam reflectance measurement (FBRM) was carried out to monitor in situ the dissolution as a function of time. FBRM uses the measured particle chord lengths and their corresponding count rates to quantify the decreasing particle size in the course of dissolution. The working principle of the FBRM device (Lasentec D 600 L-C22-K, Mettler Toledo Ltd, Australia) and the detailed experimental procedure have been described by Fang, Selomulya, and Chen (2010). In brief, 0.500 g of sample were added on the surface of 25 °C warm water that was contained in a flat 250 ml beaker. The FBRM laser probe was immersed into the water at a well-defined location and an angle of 45°, and a magnetic stirrer operated at 900 rpm. The measurements had been executed for 20 min with data collection intervals of 2 s. The FBRM measurements were conducted in triplicate with three powder samples that were collected for about 10 min each after a steady column temperature profile had been obtained.

Second, a modified approach of the Niro Analytical Method No. A5a (GEA, 2005) was used to determine the powders' wettability. The wettability, expressed in time in seconds, defines a dried powder's ability to penetrate a still water surface. For this, 0.5 g sample were passed through a funnel onto the quiet surface of Milli-Q purified water (25 °C), which had been filled into a 100 ml measuring cylinder up to the 90 ml mark. A camera (DCR-HC36, Sony Corp, Japan) recorded the wetting process from the time of powder addition until the last particle had overcome the water surface tension and consequently sunk down. The wettability study was run in duplicate with two powder samples that were collected for about 10 min each during the same spray drying run at 100 and 110 min after a steady column temperature profile had been established.

2.3.8 Peroxide value analysis for oxidative stability study

The oxidative stability of the fat contained in the spray-dried particles of different carrageenan concentrations was compared by means of their peroxide values at intervals of seven days. The powders were stored for 35 days at accelerated storage conditions of 33 °C and ambient air in a drying oven. The peroxide value measurements were carried out following the guidelines given in the Official Methods of Analysis by the Association of Official Analytical Chemists (AOAC, 1990). In short, 2 g of sample were swirled for 30 s in 30 ml of a freshly prepared acetic acid (Univar Inc, USA)/chloroform (Merck KGaA, Germany) mixture (3:2 by volume) that contained 1 ml of a saturated potassium iodate (Sigma-Aldrich Pty Ltd, Australia) solution. The mixture was then kept in dark environment for 5 min prior to addition of 30 ml of water. Subsequently, titration with a 0.002 M sodium thiosulfate (Sigma-Aldrich Pty Ltd, Australia) solution was conducted under stirring until the yellow colour had disappeared. Lastly, 1 ml of an aqueous one per cent starch indicator solution was added and it was further titrated under stirring until the blue colour had vanished. For each set of measurements, a blank control without powder was also carried out. The peroxide value $PV$ in [milliequiv. peroxide/kg fat] was calculated by the following expression:

$$PV = S \cdot N \cdot 1000/m_{wa}$$

where $S$ was the blank corrected titration volume in [ml], $N$ the normality of the sodium thiosulfate solution in [mol/l] and $m_{wa}$ the mass of fat comprised by 2 g of sample in [g]. The analysis was run in duplicate with samples that were collected for about 40 min each during the same spray drying run at 120 and 160 min after a steady column temperature profile had been reached.

3 Results and discussion

3.1 Impact of $\kappa$-carrageenan content on emulsion properties

3.1.1 Stability of the model emulsions

The preparation of the model emulsions, as described in section 2.1, resulted in complete dissolution of all components. The good water solubility of $\kappa$-carrageenan in comparison to the less charged $\lambda$ and $\iota$ forms (Langendorff et al., 2000) permitted its dissolution at a relatively low temperature, which can mean better cost efficiency in industrial applications and the avoidance of protein denaturation leading to reduced emulsion stability (Målqvist-Fureby et al., 2001).

Turbiscan emulsion stability measurements (Fig. 2) were performed to evaluate the capability of $\kappa$-carrageenan to alter the stability of the milk model emulsions at different concentrations as an indicator for the network strength between the oil and water phase. The homogeneity and stability or instability of the emulsions was evaluated by analysing the Turbiscan backscattering data over sample height as a function of time. The more precisely the scans at different times overlapped, the more stable were the emulsions. The low backscattering values at a sample height of less than 0.6 cm and above 6 cm for even the most stable samples were ascribed to light scattering at the glass bottom of the measurement cell and more light transmission at the very sample top due to the meniscus curvature.
The emulsions without carrageenan and 0.1% w/w carrageenan were least stable. They featured an increasing backscattering percentage over time throughout the sample height due to larger fat globules as a result of flocculation, and displayed a
concomitant appearance of cream at the surface, as was observed from the more pronounced backscattering at the sample top (height > 6 cm) in comparison to the other samples.

The flocculation could be inhibited by the addition of more polysaccharide. According to the width of the backscattering bands, the stability of the emulsion with 0.2% w/w carrageenan was improved in comparison to lower concentrations and greatest stability was reached for 0.3–0.4% w/w carrageenan. This indicated that the λ-carrageenan bonded onto the protein of the fat globule membranes and thus stabilized the oil/water interface by preventing coalescence of the fat globules due to their increased electrostatic repulsion. There might have been an electrostatic attraction between carrageenan and whey protein, as suggested by Stone et al. (2012). More often, though, the stabilization of milk emulsions has been attributed to interaction between carrageenan and casein (Dickinson, 1998; Ga, Reginier, & McClements, 2005; Langendorff et al., 2000), with the latter representing 80% of the protein contained in bovine milk. The pH values of the milk model emulsions were measured to be in the range of 6.44 ± 0.03, exceeding the casein's isoelectric point of 4.6. While the casein thus carried a negative net charge, the κ-casein molecules still featured positive amino acid residue regions, as generally believed (Snoeren, Payens, Jeunink, & Both, 1975). This allowed absorbance of the negatively charged sulphate groups of the carrageenan at the positively charged patches of the membranes that surrounded the fat globules. The complexation increased the overall charge of the fat globule membranes towards more negative values. Dalgleish et al. (1988) found from ζ-potential measurements using micro-electrophoresis that the negative charge density on casein/λ-carrageenan complexes in strongly diluted skim milk approximately doubled with very low carrageenan contents from about 0.001 to 0.01% w/w. Higher concentrations lead to a further, though less sharp increase in charge density within the range investigated (maximum of approximately 0.02% w/w). The flattening of the charge curve at greater carrageenan concentrations was ascribed to the cationic protein surfaces reaching saturation coverage, and it was presumed that this would have led to cross-linkage and depletion flocculation if the total solid content had been higher.

In agreement with the work by Dalgleish et al. (1988), in the present study further carrageenan addition (from 0.4 to 0.5% w/w) resulted in physical emulsion destabilization. The backscattering profile of the emulsion with 0.5% w/w carrageenan resembled the one of 0.2% w/w, being more constant over time than for 0 and 0.1% w/w. However, the emulsion was less stable than for 0.3 and 0.4% w/w, which presumably was as a result of depletion flocculation upon saturation surface coverage of the positively charged protein areas (see Appendix B for a supporting theoretical calculation of the saturation concentration). Depletion flocculation is often observed in colloidal systems when the droplets of the dispersed phase are surrounded by an adsorbed layer of polymer, which simultaneously exists in excess inside the continuous phase. As discussed by Langendorff et al. (2000), if the carrageenan concentration exceeds the saturation level to a certain degree, the free carrageenan will repulse the carrageenan-covered surfaces of the fat globules and protein aggregates and will consequently induce coalescence of the lipid phase.

The Turbiscan stability results were compared with the corresponding microstructures of the freshly prepared emulsions as visualized by the microscopic images in Fig. 2. The emulsion with 0.5% w/w carrageenan had relatively large patches of darker shade, suggesting phase separation throughout the emulsion with the dark patches being interconnected areas of flocculated fat globules. These patches were not observed at concentrations of 0.3–0.4% w/w carrageenan, where the microstructure appeared most homogeneous amongst all samples. Here only a few dark spots were observed, because the majority of the fat globules did not coalesce and thus were so finely dispersed inside the continuous phase (the brighter, grey regions in the images) that they were not visible under the microscope at the given magnification. In comparison, at lower polysaccharide contents the appearance of the emulsion microstructure was more heterogeneous with substantially more and larger dark spots, probably due to the formation of more and larger clusters by coalescence of fat globules. These most likely further coalesced over time, leading to the observed creaming. It was confirmed that the visual homogeneity of the microstructure correlated with the emulsion stability by tracking the changes over several days in an emulsion (0.3% w/w carrageenan content), which was stored at 6 °C (Figure A.1). While being relatively homogeneous initially, after the first 24 h larger coalesced lipid globules became visible and after ten days some grey patches had eventually emerged, similar in their appearance to what was observed for the fresh model emulsion of 0.5% w/w carrageenan content. It can thus be concluded that the microstructure images supported the Turbiscan stability results.

3.1.2 Fat globule size distribution and shear viscosity of the model emulsions

First and foremost, Fig. 3a compares the impact of the λ-carrageenan concentration on the size distribution of the fat globules together with the free casein micelles inside the original, untreated model emulsions. At the lowest polysaccharide concentration of 0.1% w/w, the fat globules featured maximal size with a mean diameter of 1.09 ± 0.0008 μm, which was slightly larger than in case of the carrageenan-free model emulsion. This observation was explained by bridging flocculation, where the polysaccharide chains are only loosely adsorbed on the protein of the fat globule membranes and hence may develop bridges between the fat globules, leading to physical instability. Such a bridging flocculation has also been reported for other emulsions at low polysaccharide concentration, such as by Serfert et al. (2013) for a fish oil/β-lactoglobulin emulsion at 0.05–0.15% w/w pectin concentration and by Dickinson and Pawlowsky (1997) for a n-tetradecane/bovine serum albumin emulsion at 0.001–0.04% w/v carrageenan concentration. In the present study, doubling the λ-carrageenan concentration to 0.2% w/v prevented bridging flocculation and thus resulted in a sharp decline in fat globule size. The globule size was further reduced by adding more carrageenan of up to 0.4% w/v, although the mean volumetric diameters for 0.3 and 0.4% w/v only differed slightly. A minimal fat globule size of 0.66 ± 0.0019 μm in diameter was thus achieved. This minimum coincided with the previously described saturation surface coverage concentration and an establishment of optimum emulsion stability at 0.3–0.4% w/v carrageenan (Appendix B and section 3.1.1). The physical stabilization with carrageenan prevented coalescence of the fat globules. As can be seen in Appendix A (Figure A.2), the volumetric size distribution of the emulsions was bimodal, consisting of two main peaks at approximately 0.2 and 1.5 μm. The first peak of the fat globule size distribution overlapped with the peak of the free casein micelles with their aforementioned volumetric mean diameter of 0.14 μm. The first peak was not representing the casein micelles alone, because its volume was closely related to the volume of the second peak as influenced by the carrageenan content. From 0.1 to 0.3% w/v, the volume of the second peak decreased more and more (decrease in height from approximately 6.5%, 4.5% and 3.5%). The mass of the fat phase was conserved with a corresponding increase in volume of the first peak (increase in height from approximately 5.5%, 8.7% and 9.6%). This occurred because coalescence of the smaller fat globules was inhibited by greater electrostatic repulsion due to λ-carrageenan adsorption. Comparing the emulsion of 0.1% w/v with the carrageenan-free emulsion, the size distribution shifted towards the larger fat globules of the second peak due to bridging flocculation.
Additionally, Fig. 3 also illustrates the changes in fat globule size during film disintegration in the two investigated atomization nozzles. In both cases and independent from the carrageenan concentration, the fat globules did not coalesce during atomization, but underwent a decrease in mean diameter by 6% in average. The mechanical stress inside the nozzles caused a break-up of some of the larger fat globules, as can be deduced from a slight reduction of the second peak in all atomized emulsions in comparison to the size distribution of the original emulsions (see Figure A. 2).

Aside from the observed reduction in droplet size of the dispersed phase, hydrocolloids can primarily enhance emulsion stabilities by means of their thickening effect. Despite \( \lambda \)-carrageenan being non-gelling, the dynamic shear viscosity of the model emulsions increased considerably with carrageenan content, particularly in the range of 0.2–0.4% w/w (Fig. 3b). An increase in carrageenan concentration to 0.5% w/w lacked a further appreciable rise in shear viscosity. The increasing shear viscosity with higher carrageenan content presumably was a consequence of the absorbance of carrageenan on the milk fat globule membranes. This lead to bridging flocculation at low carrageenan concentration and to electrostatic emulsion stabilization at higher concentrations until the saturation level of the protein surface areas of the fat globule membranes and casein micelles had been reached at about 0.4% w/w carrageenan content. Variation of the shear rate revealed non-Newtonian, shear thinning behaviour, being more distinct at greater carrageenan concentrations. As such, the viscosity under extensional stress was not computable from the shear viscosity, but had to be determined experimentally.

### 3.1.3 Impact of fat and carrageenan content on shear and extensional viscosities

It was hypothesized that the extensional viscosity can be a crucial factor of influence on the atomization behaviour of emulsions during spray drying. The atomization technique of the microfluidic nozzle employed in this study was not based on shear stress, but it imposed a normal stress on the emulsion jet via a sinusoidal contraction of the orifice. Fig. 1 visualizes the similarities between the microfluidic jet atomization and the acoustically-driven microfluidic extensional viscometry. In either case, the emulsion film was forming a fine thread that thinned more and more with distance from the nozzle orifice or with measurement time, respectively. At some point, depending on the viscous forces, excess surface energy was then reached, causing the thread to break up into an individual droplet to regain minimal surface energy. To better understand the impact of a dispersed fat phase and the emulsion stabilization with carrageenan, the extensional and shear viscosities of selected emulsions were compared (Table 1). The extensional viscosities were determined according to Equation (2) from the data illustrated in Appendix C (Figure C. 1).

**Table 1** Rheological impact of fat phase and \( \lambda \)-carrageenan: composition, dynamic shear viscosity (at shear rate of 199.1 s\(^{-1} \)) and uniaxial extensional viscosity of a low-fat emulsion without \( \lambda \)-carrageenan, a regular-
fat emulsion without λ-carrageenan and similar fat content to the standard model emulsions, and a standard model emulsion with 0.3% w/w λ-carrageenan.

<table>
<thead>
<tr>
<th>Carrageenan content [% w/w]</th>
<th>Fat content [% w/w in d.m.]</th>
<th>Solid content [% w/w]</th>
<th>Shear viscosity [mPa·s]</th>
<th>Extensional viscosity [mPa·s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>20</td>
<td>3.75 ± 0.07</td>
<td>25.9 ± 1.5</td>
</tr>
<tr>
<td>0.3</td>
<td>31.1</td>
<td>20</td>
<td>3.17 ± 0.02</td>
<td>11.5 ± 0.8</td>
</tr>
<tr>
<td>3.17 ± 0.05</td>
<td>0.02 ± 0.02</td>
<td>30.7 ± 2.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The low-fat emulsion with a lipid content of only 0.3% w/w in d.m. and the regular-fat emulsion with a fat content similar to the standard model emulsions (31.1% w/w in d.m.) were compared to evaluate the impact of the fat globules. Their presence caused a strong decrease in extensional viscosity to less than half the value of the fat-free emulsion (from 25.9 to 11.5 mPa·s). There was also a drop in dynamic shear viscosity, which, however, was marginal by comparison with 15% from 3.75 to 3.17 mPa·s. The sharp decrease in extensional viscosity provides an explanation for the reduction in milk droplet size with higher fat content upon atomization with a microfluidic jet nozzle as observed in a previous study (Foerster et al., 2016a), where similar emulsions were investigated. While the regular-fat emulsion had a solid content of 20% w/w in the aforementioned study, the low-fat emulsion contained only 14% w/w solids and thus featured a lower shear viscosity (2.4 mPa·s) than the regular-fat emulsion. Therefore, consideration of the shear viscosities could not explain the measurement of smaller droplet sizes in the presence of fat, as a greater viscosity typically favours the formation of bigger droplets (Lefebvre, 1989). In light of Table 1, not only the shear viscosity but in particular the extensional viscosity needs to be taken into account to describe the droplet disintegration mechanism during atomization in spray drying applications. A perforation mechanism induced by the fat globules is proposed to explain the observed sharp drop in extensional viscosity with the existence of a significant lipid content. The intrinsic milk emulsion stability might be locally reduced along the oil-water interfaces formed by the fat globules. With more fat globules being dispersed throughout the regular-fat emulsion, the emulsion became perforated by these areas of lower stability. During atomization under elongational stress, the film hence disintegrated preferably along the fat globules, following the lowest viscous resistance, figuratively speaking, like the ‘zipper of a jacket’. Such a perforation mechanism had already been briefly discussed several decades ago, when Dombrowski and Fraser (1954) and Zakarian and King (1982) undertook photographic studies of the disintegration of fan-shaped, flat liquid sheets that were generated with a single-hole fan-spray nozzle. The films broke earlier, that is at greater film thickness, in the presence of a dispersed oil phase. Furthermore, recent investigations reported that during the spray drying of milk emulsions surface layers of fat existed on the droplets immediately after atomization for various kinds of atomization techniques: microfluidic jet nozzles of different orifice size, pressure swirl nozzles of different orifice size and operated at different feed pressures, and droplet generation with a micro-volumetric syringe (Foerster et al., 2016a; Foerster, Gengenbach, Woo, & Selomulya, 2016b; Wu et al., 2014). A film disintegration localized along the dispersed fat phase would explain why the droplet surfaces were covered by fat as soon as individual droplets had been formed. If the emulsion preferably breaks up along the interface of the fat globules, the fat globules were immediately present on the surface of the formed droplets, presumably in form of a more or less consistent monolayer of fat globules that might either rupture or stay intact until completion of the spray drying process. In view of that, it was anticipated that the amount of surface fat on spray-dried milk particles should be reducible by decreasing the size of the fat globules inside the emulsions with the addition of λ-carrageenan. In addition, the emulsion stabilization with carrageenan was hoped to shift the disintegration mechanism away from the fat globules.

Investigation of the impact of λ-carrageenan (0.3% w/w) on the viscosity of the standard regular-fat emulsion showed that the carrageenan increased the extensional viscosity significantly (Table 1). The milk model emulsion with 0.3% w/w λ-carrageenan featured an extensional viscosity of 30.7 mPa·s, which was by a factor of 2.7 greater than the one of the polysaccharide-free emulsion of identical fat content. The loss in extensional viscosity due to addition of the lipid phase was consequently overcome and the extensional viscosity was even greater than the one of the low-fat emulsion. Accordingly, the diameter of the monodisperse droplets immediately after atomization was slightly larger for 0.3% w/w (122 ± 2 μm) in comparison to the carrageenan-free emulsion droplets (115 ± 2 μm). This indicated that the stabilized emulsions might have less preferably disintegrated along the dispersed fat phase.

3.2 Impact of λ-carrageenan content on the spray-dried particles' properties

3.2.1 Chemical surface composition

The amount of surface fat on the spray-dried particles was significantly reduced at certain carrageenan concentrations, as determined by surface fat extraction (Fig. 4a). The surface fat amounted to 4.7% for 0.3% w/w carrageenan, in contrast to 13.8% for the carrageenan-free powder. The extent of surface fat formation during spray drying was approximately inversely proportional to the emulsion stability, as the lowest amount was observed at intermediate carrageenan concentrations. Being at 0.2-0.3% w/w, this was slightly offset from the optimum emulsion stability and fat globule size values at 0.3-0.4% (Fig. 2 and Figure 3a). In agreement with this finding, a more efficient fat encapsulation with decreasing fat globule size inside the emulsions to be spray-dried, indicating greater emulsion stability, was also reported in other studies (Jafari et al., 2008; Sarkar, Arfsten, Golay, Acquistapace, & Heinrich, 2016). A strengthened network between the lipid and aqueous phases through stabilization of the milk fat globules by adsorption of λ-carrageenan, as also reflected by the substantial increase in extensional viscosity, possibly led to a reduction of the in the section 3.1.3 discussed perforation mechanism. As the network along the fat globules was reinforced, it is believed that the emulsion film disintegrated less preferably along the phase interfaces and thus a smaller amount of fat emerged on the surfaces upon droplet formation. The emulsion stability seemed to entail a decisive influence, since the fat proportion at the surface went up again to 10.0% for the emulsion with the highest carrageenan concentration of 0.5% w/w, which presumably was subject to depletion flocculation. Other than that, the reduction in fat globule size (discussed in section...
3.1.2) in the stabilized emulsions possibly contributed directly to the observation of less surface fat. From the fat extraction data, the thickness of the surface fat was roughly estimated under the assumption of a continuous fat layer that had the same composition as measured for the very surface by XPS (albeit this is not the case in reality). Surface fat thicknesses of 1.0, 0.9, 0.4, 0.4, 0.6 and 0.6 μm were calculated for the respective powders from emulsions with 0–0.5% w/w carrageenan. Interestingly, these estimations approximately corresponded to the respective sizes of the fat globules as measured inside the emulsions (Fig. 3a). This suggested that about a monolayer of fat globules, albeit presumably to some extent in ruptured form, might have been present on the spray-dried particle surfaces. This is in conformity with the proposed surface fat formation mechanism in consequence of preferred disintegration along the dispersed fat phase during atomization. In view of this, a smaller fat globule size led to less thick surface fat on the generated droplets and thus on the spray-dried particles.

![Fig. 4](image)

**Fig. 4** Impact of λ-carrageenan content of the model emulsions on the corresponding spray-dried particles’ surface composition: (a) free surface fat from extraction study, and (b) estimated XPS surface composition of the spray-dried particles for all carrageenan concentrations, of the droplets with 0.3% w/w directly after atomization and of the spray-dried particles from emulsions with 0.1 and 0.3% w/w after surface fat extraction.

XPS analysis of the surface concentration (Fig. 4b) only showed minor differences between the powder samples. As the XPS surface concentration in lactose, protein and fat on the various spray-dried powders was a linear estimation derived from the atomic concentrations, the small differences between the samples were not statistically significant. More generally, the XPS results showed that the powder consisted almost completely of fat (approximately 88-94% v/v) at their very surface and addition of carrageenan could not inhibit the creation of a dominant fat coverage along the outmost particle surface. As such, XPS analysis did not reflect the strong influence of the carrageenan content on the extent of surface fat that was revealed by surface fat extraction. Protein took up the remaining volume of the analysed surface layer, which was depleted in lactose. For 0.3% w/w carrageenan inside the model emulsion, Fig. 4b also compares the surface concentration of spray-dried powder particles with the one of the corresponding droplets immediately after atomization for 0.3% w/w carrageenan. Apparently, a surface fat layer had already been formed during atomization (77% v/v fat) and the XPS surface concentration did not change
significantly throughout the succeeding drying stage. Furthermore, it was concluded that at least some of the fat globule membranes got ruptured at the droplet surfaces by mechanical or thermal stress during atomization or drying, and the free fat could spread over wide areas of the droplet surface. If the majority of the membranes was still intact, the XPS measurement would most likely have indicated greater protein surface concentrations, because the thickness of the adsorbed casein layers in homogenized milk model emulsions are typically in the range of approximately 5–11 nm (Dalgleish, Srinivasan, & Singh, 1995; Fang & Dalgleish, 1993), which coincides with the XPS measurement depth of less than about 10 nm. Instead, as a result of the presumed membrane rupture of at least some of the fat globules at the surface, primarily the lipid phase was detected by XPS. The fat surface concentration did not reach 100% v/v, nevertheless, because not all globules got ruptured at the surface (see section 3.2.2) and some protein of the original membranes might have remained near the surface or other protein diffused to the surface during the drying stage due to its surface activity. Regarding the extent of globule rupture, the adsorbed carrageenan layers presumably had an indirect influence on the membrane stability: the membranes of the smaller fat globules, as for instance obtained by emulsion stabilization with carrageenan at intermediate concentrations, were less susceptible to rupture than the ones of the largest globules (Xu et al., 2013).

The protein and fat distributions obtained from confocal laser scanning microscopy showed that the surfaces of all spray-dried particles were covered by a fat layer (Fig. 5a and b). Moreover, the thickness of the fat surface layers strongly varied with carrageenan content. For 0% w/w, the signal of the surface fat was stronger and reached further into the inner of the particle in comparison to 0.3% w/w. The component distribution in the atomized droplets of 0.3% w/w carrageenan content appeared to already feature a hardly visible fat surface layer (Fig. 5c). The low visibility of this thin surface layer was ascribed to the limited resolution of the CLSM image and the porous surface structure of the atomized droplets after freeze-drying. The variation of the thickness of the surface fat observed from CLSM thus explained why the surface fat extraction demonstrated a strong dependence of the amount of surface fat on the carrageenan content. Additionally, as even for low surface fat contents the outmost surface was comprised predominantly by fat, the CLSM images also explained why the XPS measurements, which accounted only for the composition at the very particle surface due to the short sampling depth, detected a fat dominance at the surfaces throughout all spray-dried powders and on the atomized droplets.
Fig. 5 CLSM images of distribution of protein (signal from Fast Green FCF visualized in green) and fat (signal from Nile Red in red): (a,b) cross-section of spray-dried particles with λ-carrageenan contents of 0 and 0.3% w/w, and (c) cross-section of atomized droplet (after flash-freezing and freeze-drying) with 0.3% w/w λ-carrageenan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
3.2.2 Particle morphology and porosity

In addition to the chemical surface composition, also the particle morphology and porosity can affect the functional properties of milk powder. SEM investigation showed that the particle surfaces obtained from emulsions without carrageenan or with low concentration thereof featured convex bumps of 2-4 μm in diameter (Fig. 6a and b). With more polysaccharide, the particle surfaces became smoother (Fig. 6d and e). Comparing the particle surfaces at 0.1% w/w before and after surface fat extraction (Fig. 6b and c), the convex bumps had disappeared after the extraction process and instead concave dimples of approximately similar size and distribution density remained. It was hence concluded that the bumps consisted of fat and were formed during spray-drying under coalescence of fat globules on the drying particles’ surfaces. Accordingly, it appears that not all of the fat globule membranes that were located at the droplet surfaces underwent rupture (compare to section 3.2.1). With the addition of λ-carrageenan, the negative charge of the fat globules’ membranes rose and thus coalescence of fat globules on the surfaces was inhibited and less to no bumps were observed. Alternatively, the bumps might have been formed by protein aggregates. Comparing the CLSM images before and after the drying stage (Fig. 5b and c), it appears that protein clusters had been formed during drying or storage, at least in the protein-rich inner of the particles. It is well known that in spray-dried high-casein powders the casein has a tendency to form networks of interconnected micelles with low solubility in aqueous media (Crowley, Kelly, Schuck, Jeantet, & O’Mahony, 2016; Schuck et al., 2007). Also, it has been suggested in literature that, at elevated temperature of sufficient height and duration, whey protein exposes hydrophobic amino acid residues that then undergo linkage with casein micelles, leading to hydrophobic aggregate formation (Corredig & Dalgleish, 1999). The reported protein aggregate formation occurred for particles that were very high in protein, whereas in the present study the particle surfaces were dominated by fat. It is hence doubtful whether here such hydrophobic protein aggregates could have developed at the particle surfaces.

Although the external surfaces of all powder samples were non-porous, the internal cross-sections contained macro-porous structures, which were more pronounced at lower carrageenan content (Fig. 6f-i). It is hence believed that the strengthening of the emulsion network by addition of carrageenan translated into denser particles upon spray-drying. This also explains why the spray dried particles from emulsions with 0 and 0.3% w/w carrageenan were of similar size (91 ± 4 and 90 ± 3 μm in diameter, respectively), despite the atomized droplets being about 7 μm larger for 0.3% w/w (see section 3.1.3). Thus, both the amount of surface fat as well as the particle porosity are to be taken into account when discussing the impact of carrageenan on the functional powder properties.

3.2.3 Functional powder properties

Fig. 7a illustrates the results of the dissolution and wettability studies. At the end of the FBRM dissolution test, the particle size, as represented by the median chord length, was generally larger for any λ-carrageenan concentration in comparison to the carrageenan-free powder. In the range of 0.1-0.5% w/w, there was a local minimum in particle size at an intermediate carrageenan content of 0.3% w/w. The curves of chord length over full dissolution time were in agreement with this and are provided in Appendix D (Figure D. 1). The wettability study revealed that the samples containing 0.3% w/w polysaccharide completed the penetration of the water surface the fastest in a time of about 17 min. The wetting times of the other samples (0, 0.1,
0.2 and 0.4% w/w), defined as total time required for the last particle of each sample to penetrate the water surface, did not vary significantly from each other, lying between 23 and 25 min. No wetting time for powder samples obtained from spray-drying of an emulsion with 0.5% w/w carrageenan are displayed in the chart, because the majority of those particles had still not penetrated the water surface after 1 hour. The results of the wetting time were in line with photographic monitoring at 20, 60, 120 and 300 s, which also showed a superior wettability for 0.3% w/w and a particularly limited wettability for 0.5% w/w (Figure D. 1 in Appendix D). For interpretation of these results, the dissolution and wettability studies need to be interpreted in relation to each other. The powder wettability seemed to influence the powder solubility, as the wettability curve over carrageenan content was similar in shape to the dissolution curve with a minimum at 0.3% w/w carrageenan. The minimal value in chord length and wetting time at 0.3% w/w is believed to be a consequence of the powders’ different surface fat contents. As the amount of hydrophobic surface fat decreased from 0 to 0.3% w/w of added carrageenan, the thereby improved wettability in aqueous medium counteracted, to some extent, the detrimental impact of the declining porosity that was observed from SEM imaging. Presumably as a result of lower particle porosities with greater carrageenan content, the powder dissolution rate was lowered by any carrageenan content in comparison to the polysaccharide-free powder. Therefore, an unwanted deterioration of the powder rehydration behaviour was caused by emulsion stabilization with carrageenan.

Furthermore, the internal porosity of the powders also affected their peroxide values (Fig. 7b). These were used as a measure of the extent to which the lipid’s unsaturated fatty acid chains had undergone primary oxidation. Directly after spray drying (0 days), no difference in the degree of oxidation was observed between any sample (colour of the initial analyte solutions was already similar to the blank tests prior to titration). Apparently, the fatty acids had not been appreciably oxidised during emulsion preparation or spray drying for any powder sample, and the peroxide values hence were approximated as being 0 milliequiv/kg. The most significant changes in peroxide value and the most distinct differences between the individual powder samples were found to occur during the first seven days. The greater the carrageenan content of each sample was, the less strongly increased the corresponding peroxide value, indicating an improved oxidative stability by the addition of carrageenan. This was explained by the decreasing internal powder porosity at higher carrageenan concentration, which impeded oxygen diffusion inside the particles. Therefore, the peroxide values of the 0.5% w/w powder samples were by far the lowest (6–17 milliequiv/kg) over the whole storage study. It should be noted that the difference in powder dissolution rate and wettability might have also affected the peroxide value measurement itself by influencing the accessibility of the titrant and the sample.

![Alt-Text: Fig. 7](image-url)
solution into the particles. In addition, a smaller amount of surface fat most likely supported lower peroxide values, too. For instance, there was a considerable difference in the peroxide values at the end of the storage study (35 days) between powders with a carrageenan content of 0 or 0.1% w/w (approximately 33 milliequiv/kg) and 0.2-0.4% w/w (27-22 milliequiv/kg). This corresponded well with the sharp drop in extracted surface fat from 0.1 to 0.2% w/w (Fig. 4a). That the lipid contained in particles can be protected from a high rate of oxidation by reducing the amount of surface fat was previously demonstrated by Granelli et al. (1996).

4 Conclusions

This study gave an understanding about the effect of λ-carrageenan, a linear sulphated polysaccharide, on milk model emulsions in terms of stability and disintegration behaviour during spray drying for the purpose of improved milk powder properties by a reduction of surface fat. From investigation of emulsions at different carrageenan concentrations, it was learnt that coalescence of the dispersed lipid phase can be inhibited by adsorption of λ-carrageenan’s sulphate groups on the milk fat globule membranes and a consequent increase in negative charge of these oil-water interfaces. Optimum electrostatic emulsion stabilization and minimum fat globule size were obtained within a certain range of carrageenan concentrations (0.3-0.4% w/w) for the given model emulsions, which agreed with a theoretical estimation of the saturation coverage of the cationic casein surface area. The emulsion stabilization translated into a considerably thinner surface fat layer on the spray-dried particles. The fat in powders obtained from a carrageenan-free emulsion consisted of 13.8% free surface fat, in contrast to only a third of this (4.7%) for a carrageenan content of 0.3% w/w. These fat extraction results were confirmed by confocal laser scanning microscopic images. It was concluded that the emulsion stabilization with carrageenan possibly reduced the proposed perforation mechanism along the dispersed fat phase and decreased the volume of the fat globules that appeared at the droplet surface through this disintegration process, contributing to the observation of less surface fat at intermediate carrageenan concentrations. Rheological data suggested that during atomization the milk emulsions preferably disintegrated along the dispersed fat globules. The extensional viscosity was very sensitive to the addition of oil and λ-carrageenan. While the addition of a significant fat phase at constant total solid content resulted in a decrease from 25.9 to 11.5 mPa s, the presence of carrageenan (0.3% w/w) averted this trend by raising the extensional viscosity up to 30.7 mPa s. The new insights into the connection between emulsion properties, atomization behaviour and resulting surface composition of the corresponding particles is of importance for industrial spray drying applications, since surface fat coverage affects the functional powder properties detrimentally. In light of these results, and as carrageenan is already being used in commercially available dairy drinks, the addition of carrageenan into milk emulsions for reduction of the powder surface fat coverage upon spray-drying seems to be feasible from an economical and consumer point of view. Besides a reduction in surface fat, the emulsion stabilization with λ-carrageenan also induced a decrease in internal particle porosity. With regard to the rehydration behaviour in water, a trade-off was noted between the positive impact of reduced surface fat and the negative influence of a greater particle density with carrageenan. Since the effect of increased density was more dominant on the water solubility, methods to avoid a loss in powder porosity should be explored in future studies for the purpose of ideal functional milk powder properties in industrial applications. Yet, the higher powder density appeared to also have a favourable effect, as the powder’s oxidative rancidity after storage was reduced with greater carrageenan content. In a future study, it could be investigated how the improved emulsion stability and increased extensional viscosity by addition of carrageenan impacts the disintegration behaviour and fat encapsulation efficiency in spray drying techniques that are commonly applied in industrial milk powder production. The charge density in emulsions containing skim milk powder and different λ-carrageenan concentrations could be investigated by ζ-potential measurements to also study how the electrostatic carrageenan/casein interaction is influenced by a variation in sodium or calcium cation concentrations.

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Appendix E. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodhyd.2017.04.005.

Appendix A. Fat globule size distributions and changes in emulsion microstructure over time
Fig. A1 Emulsion homogeneity as a function of time: microscope images of the microstructure of a model emulsion (0.3% w/w carrageenan) that was stored at 6 °C.
Fig. A2 Volumetric size distribution in model emulsions of different carrageenan contents: in the original emulsions (first column) and after atomization in a microfluidic jet nozzle (second column).
Appendix B. Estimation of the saturation coverage of the protein surfaces by carrageenan

The emulsion stability investigation (section 3.1.1) indicated that saturation coverage of the cationic surface areas of the protein of the casein micelles and around the fat globules, and as such optimum stability that deteriorates again for higher concentrations presumably due to depletion flocculation, occurred at approximately 0.4% w/w carrageenan. This value was about 30 times greater than the values reported by Dalgleish et al. (1988). This seemed reasonable, because the emulsions investigated in the present study contained a much higher fat content, leading to a greater protein surface area around the lipid globules, and a significantly higher concentration in protein (5.4% w/w, in comparison to about 0.025% w/w). Theoretical calculation, comparable to (Dalgleish et al., 1988), was undertaken to provide further supporting evidence that flocculation occurred due to excess carrageenan at a carrageenan concentration above 0.4% w/w. For this consideration, it was supposed that at saturation level the positively charged regions of the protein at the surface of the fat globules and the casein micelles were completely covered by a monolayer of carrageen. The fat globules featured a volumetric mean diameter of around 650 nm (Fig. 3a), and their surface area was thus about 0.64 m²/g emulsion for the given lipid concentration inside the model emulsions. Estimating a surface casein concentration on the fat globules of 1.50·10⁻³ g/m² according to analyses by Srinivasan, Singh, and Munro (1996) of a soya oil/caseinate emulsion homogenized at high pressure, the non-adsorbed amount of casein was 5.30·10⁻² g/g emulsion. Together with the volumetric mean diameter of the casein micelles being previously measured as approximately 140 nm, this amounted to a surface area of casein micelles of about 1.82 m²/g emulsion. Hence, the combined surface area available for carrageenan adsorption was 2.46 m²/g emulsion. The carrageenan molecules were assumed to span an area of 6.0·10² m²/g as adapted from Dalgleish et al. (1988). This meant an absolute surface area of 0.60, 1.20, 1.80, 2.40 or 3.00 m²/g emulsion held by the polysaccharide molecules for respective concentrations of 0.1, 0.2, 0.3, 0.4 or 0.5% w/w. Consequently, the surface area of casein micelles and fat globules was approximately commensurate with the present carrageenan surface area for a carrageenan content of 0.4% w/w, and at 0.5% w/w there was more carrageenan inside the emulsion than could undergo electrostatic interaction with the positively charged protein regions. The theoretical calculation thus agreed well with the observed emulsion stabilities (section 3.1.1) and the results about the fat globule sizes as a function of carrageenan concentration (section 3.1.2). The calculated saturation surface coverage corresponded to an adsorbed carrageenan amount of 1.62·10⁻³ g/m².

Appendix C. Time curves of neck diameter during extensional viscosity analysis
Fig. C1 Extensional viscosity measurements: bridge neck diameter over time for low-fat emulsion, regular-fat emulsion, regular-fat emulsion with carrageenan (0.3% w/w) and pure water for comparison.

alt-text: Fig. C1
Appendix D. Full results of FBRM dissolution study and images taken during wettability study
Fig. D1 Impact of $\lambda$-carrageenan concentration on powder dissolution rate: median diameter of the particles’ chord lengths as a function of dissolution time.
Fig. D2 Impact of λ-carrageenan concentration on powder wettability: photographs taken at 20, 60, 120 and 300 s after addition of powder onto the water surface.

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The following is the supplementary data related to this article:

Multimedia Component 1

Video 1 Thinning emulsion bridge during microfluidic extensional viscometry of a milk model emulsion with 31.1% w/w fat in d.m. and no carrageenan.

Appendix E. Supplementary data

The following are the supplementary data related to this article:

Multimedia Component 2

Video 2 CLSM stack animation of protein (green) and fat (red) distribution inside a spray-dried particle from a carrageenan-free model emulsion from the top to about its middle in 1 μm steps.

Multimedia Component 3

Video 3 CLSM stack animation of protein (green) and fat (red) distribution inside a spray-dried particle from a model emulsion with 0.3% w/w λ-carrageenan from the top to about its middle in 1 μm steps.

Graphical abstract
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