Spray Dried Milk-Protein Stabilized Emulsions with High Oil Content

A thesis presented in partial fulfilment of the

requirements for the degree of

Doctor of Philosophy

in

Food Technology

At
Riddet Institute, Massey University
Palmerston North
New Zealand

Amit Taneja

2016

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Abstract

This study explores the behaviour of oil droplets in milk protein-stabilized emulsions during spray drying. The impact of preheat treatment on the stability of oil droplets during drying in milk protein-stabilized emulsions in maltodextrin was also observed, using a variety of techniques such as particle size analysis, various microscopy techniques and sodium dodecyl sulphate polyacrylamide gel electrophoresis. In the last section of the study, the stability of the powdered emulsions was investigated against oxidative deterioration when soybean oil was replaced with fish oil in the emulsion formulation.

The results showed that spray drying and redispersion of the powdered emulsions in water (at similar total solids content) caused a shift in the droplet size distribution to larger values for all emulsions made using low concentration of whey protein isolate or sodium caseinate (0.5−2.0%, w/w w.b.), in comparison with their respective parent emulsions. However, the droplet size distribution was affected only very slightly by spray drying when the protein concentration was above 2.0% (w/w). This minimum concentration of protein that was required to produce emulsions that were stable during the spray drying process was 3.0% (w/w) for the emulsions prepared using aggregated milk protein products as compared with 2.0% (w/w) for the NaCas- and WPI-containing emulsions.

It was suggested that the amount of unadsorbed protein in the bulk phase of the parent emulsions play a crucial role in stabilizing the oil droplets during spray drying. When the surface of the oil droplet is saturated with protein molecules and the bulk phase of the emulsion has sufficient unadsorbed protein, the oil droplet is

stable during drying. However, for emulsions with a low concentration of unadsorbed protein in the bulk phase (≤ 1.0% for WPI or NaCas emulsions), protein molecules could potentially migrate from the surface of the oil droplet to the air–water interface, causing “gaps” in the oil droplet interface and leading to coalescence and/or bridging flocculation.

Emulsions containing low levels of maltodextrin showed marked coalescence during spray drying and redispersion even at a WPI concentration of 10.0% (w/w). Above a critical concentration (12.0%, w/w), maltodextrin appeared to stabilize proteins at the interface and provide adequate rigidity to the matrix perhaps by forming a glass, under the drying conditions.

In whey protein-stabilized emulsions made with preheat treated protein solution (above 70ºC), a shift was observed in average droplet diameter towards the larger size range, because of droplet coalescence as a result of spray drying. This was thought to probably be a result of protein aggregation in emulsions, which adversely affected the ability of proteins to stabilize the emulsion droplets during spray drying and further emphasized the crucial role of monomeric whey proteins. A reduction in the non-adsorbed monomeric whey proteins as a result of preheat treatment led to oil droplet coalescence during drying. The stability of the emulsion made with pre-heat treated whey proteins was noticeably improved when NaCas was added to the emulsion either before or after the homogenization step. This improved stability was believed to be a result of the steric effect of caseins that prevented large-scale aggregation of whey proteins.

The stability of emulsions during drying as shown by the change in the average droplet diameter before and after drying showed a negative correlation with

oxidative stability of these emulsion where soybean oil was replaced with fish oil. The protein content and preheat treatment also showed a positive impact on the oxidative stability of spray-dried emulsions.

Overall, the finding from this systematic study has advanced the understanding of the mechanisms of the stability of oil droplet during drying as well as the impact of emulsions components and processing conditions. This may help to design emulsion formulations and processes and extend the applications of milk-protein stabilized powdered emulsions with high oil content.

Acknowledgements

For me, the journey towards completing my PhD has been a very special one. I've received support and guidance from so many people during this time towards whom I'd like to express my deepest gratitude.

A special thank you goes to my supervisor, Dist. Professor Harjinder Singh, not only for his tremendous academic support, but also for giving me so many wonderful opportunities during the time I spent at the Riddet Institute. Harjinder truly has been an inspiration for me to work hard and back my abilities. I also had the pleasure of having many great discussions with Harjinder regarding my research and a few good discussions on cricket and how to fix my golf swing.

I am also very grateful Dist. Professor Paul Moughan for his continued encouragement throughout my studies. He has always inspired me to speak my mind no matter how unconventional it may sound.

I also thank, Professor Richard Archer, my co-supervisor, for always inspiring to be rigorous in my approach and providing critical evaluation during the planning of my experimental chapters.

A big thank you to Professor Jim Jones, my co-supervisor, for providing continued support and encouragement that was a driving force for me to keep pushing hard and continue my research with great enthusiasm.

Associate Professor Aiqian Ye has been the person that I go to anytime to discuss openly regarding my research. I appreciate the time that Aiqian made available for

to meet and listen patiently to any of my concerns and give honest feedback. Thank you Aiqian for being so dedicated to your role as my co-supervisor.

I want to acknowledge the contributions of the following people

- Anant Dave for training me on electrophoretic equipment and techniques.
- Sophie Gallier and Jianyu Chen for showing me how to operate the Confocal Laser Scanning Microscope at the MMIC, Palmerston North.
- Dr. Carlos Montoya for helping with the densitometry software for PAGE scanned gels.
- Chris Hall and Janiene Gilliand for their support with particle size measurement.
- Dr. Peter Zhu for helping with the GC headspace propanal analysis and measurement with peroxide value.
- Dr. Vikas Mittal for his guidance with the use of sigmaplot statistical software.
- Professor Douglas Dalgliesh for spending time with me to discuss the effects of heating on whey proteins.
- Doug Hopcroft for his assistance with sample preparation and helping me with image analysis on the Scanning electron Microscope.
- Jianyu Chen for patiently working with me to develop a modified method for transmission electron microscopy of my powder samples.

I've spent a lot of time at the Riddet Institute and even though I am not there anymore, the people that I worked with have special place in my heart. Thank you to my Riddet colleagues Ansley, Terri, Fliss, Willi, John, Shane, Sharon, Janiene, Natascha, Nok, Jack, Maria, Guilaume, Ajit, Carlos and Chris (Teddy).

A sincere and a very special thank you to the people who never stop giving, Shantanu Das, Jinita Das, Anwesha Sarkar, Preet Mohinder Singh and Jaspreet Kaur.

My family has always been there for me and understood what my research meant to me. Thank you Nancy, Anushka, Pervez and Ritu Taneja for being there for me.

Finally, but by no means least, thanks to Nimmi, my better half, and my son Arjun for their unconditional and almost unbelievable support. They are the most important people in my world. I dedicate this thesis to them.

Contents

List of Figures

List of Tables

List of Publications

Taneja, A., & Singh, H. (2012). Challenges for the delivery of long-chain n-3 fatty acids in functional foods. *Annual review of food science and technology*, *3*, 105-123.

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*(1), 15-23.

Taneja, A., Ye, A., & Singh, H. (2015). Influence of protein concentration on the stability of oil-in-water emulsions formed with aggregated milk proteins during spray drying. *Dairy Science & Technology*, *95*(3), 279-293.

Chapter 1

Introduction

Spray drying has been in use for decades for food preservation where the final moisture content of the product is reduced to <3% in order to minimise physical, chemical or biological deterioration (Gharsallaoui et al., 2007; Maher et al., 2014; Vega & Roos, 2006). In many cases, emulsions are dried into powder to extend shelf life and provide convenience to consumers (Faldt & Bergenstahl, 1996; Vega & Roos, 2006). These include foods like whole milk powder, nutritional powders (e.g. infant formula) and advanced medical nutrition products. Milk proteins are the main emulsifiers in these emulsion systems and the amount of protein required to stabilize the emulsions depends on the type of milk protein product used, the oil/fat content of the formulation and the powder properties desired in the finished products (Hogan et al., 2001; Vega & Roos, 2006; Maher et al., 2014).

Often other constituents such as low molecular weight sugars like lactose, sucrose or maltodextrin are added in the continuous phase to these types of emulsion formulation to aid drying. The physico-chemical behaviour of such emulsions during drying and upon reconstitution is affected by the properties of the added sugars. Sugars are advantageous due to their low cost, low viscosity in concentrated solutions, and the ability to produce a glass around emulsion droplets upon spray drying providing stability (Bhandari & Howes, 1999). Sugars, in combination with milk proteins such as whey protein products or caseinates, provide excellent

emulsifying properties as well as good physical properties in the resulting powders (Hogan et al., 2000; Maher et al., 2014; Vega & Roos, 2006).

The physicochemical properties of dairy powders have been subjected to extensive research in the past few decades (Buma, 1971; Bronlund & Paterson, 2004; Fitzpatrick et al., 2004; Vega et al., 2005; Kim et al., 2002; Vega & Roos, 2006). The processing parameters such as the physical configuration of the spray dryer, inlet/outlet temperatures, feed solids concentration on the final powder properties (Kim et al., 2009). However, these studies have used standard milk powders (i.e. skim milk powder, whole milk powder etc.) that are widely available in the food industry and some model systems containing milk proteins and lactose. In addition, there have been a limited number of studies on the spray drying of emulsions with high oil content (> 20% dry basis).

On the other hand, extensive research exists on milk-protein-stabilized pure emulsions and the impact on their stability as a function of milk protein type, protein concentration, heating and change in ionic environment (Dickinson 1998, 1999, 2008; Golding & Wooster, 2010; McClements, 2010; Singh & Ye, 2009). However, the impact of spray drying on such emulsions is not well understood.

This work seeks to address the above questions and understand the changes that emulsion droplets undergo during the spray drying process. The interrelationship between emulsion composition and processing on the most important physico-chemical properties of spray-dried emulsions (surface composition, the reconstitution behaviour and the stability during storage) have been investigated in detail in high oil powdered emulsions.

Chapter 4 compares the behaviour of oil droplets during spray drying and after redispersion in oil-in-water emulsions stabilized by whey protein isolate (WPI) or sodium caseinate (NaCas) at a range of protein concentrations. The effects of spray drying on the properties of the emulsions stabilized by whey protein as a function of protein and maltodextrin (MD) concentration were also explored.

Many different variations of milk protein products are now commercially available for use as food ingredients including protein products containing mainly monomeric protein as well as protein products containing aggregated protein species e.g. milk proteins concentrate (MPC) and calcium caseinates (CaCas). Although the emulsifying and adsorption properties of MPC and CaCas in oil-in-water emulsions have been well documented (Euston & Hirst 1999; Ye 2011; Ye et al., 2000), their ability to stabilize oil droplets during spray drying has not been reported. In Chapter 5, the behaviour of oil droplets during spray drying of emulsions stabilized with aggregated milk proteins has been investigated.

Chapters 6 reports on the effects of heat treatment on the behaviour of oil droplets in whey-protein stabilized oil-in-water emulsions. It is well known that the main whey proteins, β-lg and α-la, unfold (denature) from a globular conformation and aggregate upon heat treatment above 70°C (Euston, Finnigan, & Hirst, 2000). This has a marked impact on their ability to form stable emulsions and provide stability against oil droplet coalescence (Sliwinski et al., 2003). However, It has also been reported that large-scale aggregation of whey proteins is inhibited in the presence of a small amount of caseinate (Dickinson & Parkinson, 2004). In Chapter 7, the influence of the presence of NaCas on the stability of heat-treated WPI stabilized emulsions during spray drying have been explored.

There is an interest in the fortification of food products with omega-3 fatty acids (through fish oil), due to their well-known health benefits (Shen et al., 2007). However, their susceptibility to oxidation presents a major challenge. Chemical stabilisation of fish oils has been extensively researched with the use of microencapsulation techniques. Some of these microencapsulation techniques use spray drying as means of protecting the fish oil. Chapter 8 reports on the interrelationships between the factors that impact the stability of powders containing fish oil, i.e. the type and the concentration of milk proteins and maltodextrin, pre-heat treatment and oxidative stability.

Chapter 2

Review of literature

The aim of this review is to discuss the creation and stability of milk proteins stabilized emulsions and the factors affecting stability of emulsions. These factors include, protein concentration, oil concentration, pH, ionic strength and heat treatment. Furthermore, the current understanding of dehydration of milk protein stabilized emulsions by spray drying is also reviewed. The impact of emulsion composition and processing conditions of the final powder will also be discussed along with the use of spray drying for the microencapsulation of long chain polyunsaturated fatty acids.

2.1 Introduction

The principles of emulsion science and technology are widely used in the food industry to create a wide variety of commonly consumed food products. These include coffee creamers, nutritional formulae, nutritional beverages, margarines, sauces, dips, deserts, flavoured milk beverages etc. Some of these products might exist as stable emulsions as final products (flavoured milk, sauces etc.) or may have been in an emulsion format during preparation or processing (coffee creamer, nutritional formulae etc.). The physical, chemical and organoleptic characteristics (such as shelf-life, flavour, texture and appearance of the final product) can be controlled by the food manufacturers by selecting appropriate ingredient types and processing parameters (McClements, 2010).

Two main types of emulsions are primarily used in the food industry for the manufacture of commonly consumed processed foods: oil-in-water (O/W) and

water-in-oil (W/O). Milk, cream, beverages and dressings are examples of O/W emulsions. In these types of emulsions, the oil is the dispersed phase and water as the continuous medium. On the other hand, margarine and butter are examples of W/O emulsions. In this case, the oil is the medium and water is the dispersed phase as shown in Figure 2.1 (McClements, 2010).

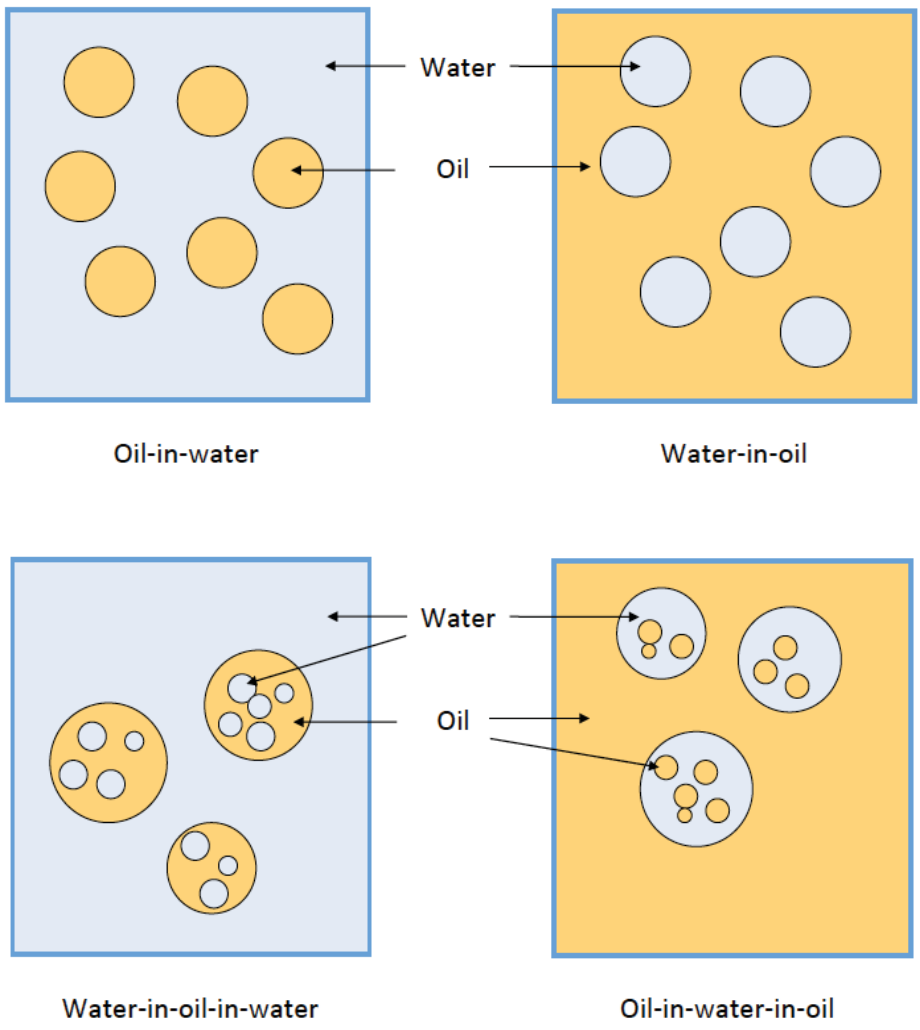


Figure 2.1 *Schematic representations of the different types of emulsion systems. Blue and yellow represent water and oil phase respectively (adapted from Emulsions & Emulsification, 2009).*

The other type of emulsion systems are known as multiple emulsions where a number of droplets are dispersed in such a way that the final dispersed phase is continuous phase for the internal dispersed droplets. These emulsions have been further classified into either a water-in-oil-in-water (W/O/W) or an oil-in-water-in-oil (O/W/O) type emulsion. The internal phase in O/W/O emulsion consists of oil whereas in W/O/W, the internal phase is water (Florence & Whitehill, 1981; Sarkar, 2010). In this review, the main focus will mainly remain on oil-in-water type emulsions. Other types of emulsions have been covered in a number of publications (McClements, 2005; Dickinson, 1992; Dickinson & Stainsby, 1982). The liquid emulsions systems are covered first, followed by dried emulsions.

2.2 Emulsion formation

As mentioned above, an emulsion system consists of a minimum of two phases that are generally immiscible liquids. Depending on the size of the droplets, emulsions can be categorized into three different types (Table 2.1). An emulsion with an average droplet size ranging between 100nm to 100μm is referred to as a macro-emulsion. These types of emulsions are generally turbid due to the larger size of the oil droplets and are generally thermodynamically unstable. On the other hand, nano-emulsions contain very small sized oil droplet ranging between 20nm-100nm. Due to the small size of the oil droplet, these emulsions are either transparent or slightly turbid and are stable against phase separation as compared to macro-emulsions. Due to the lower free energy of the water phase as compared to the oil phase, these emulsions are still thermodynamically unstable. The most stable types of emulsions are micro-emulsions with oil droplets ranging between 5nm to 50nm. These emulsions can be transparent due to less scattering of light as the particle sizes tend to be much smaller (McClements, 2010).

Table 2.1 *Different types of oil-in-water emulsions and their properties (McClements, 2010).*

Emulsion Type	Average droplet diameter range	Thermodynamic stability	Surface to mass ratio (m^2/g)	Appearance
Macro-emulsion	0.1-100μm	Unstable	0.07-70	Turbid/opaque
Nano-emulsion	20-100nm	Unstable	70-330	Transparent
Micro-emulsion	5-50nm	Stable	130-1300	Transparent

Due to the thermodynamic instability of emulsion systems, disruption may occur over time due to gravitational separation of the phases, oil droplet coalescence, partial coalescence of oil droplets and/or Oswald ripening (Dickinson, 1992; Friberg et al., 2004, McClements, 2010). Majority of the research in the field of emulsion science is therefore concerned with the long-term stability of such systems (McClements, 2010).

The preparation of oil-in-water emulsions generally takes place using a high shear equipment, such as the high pressure valve homogenizer or a high speed shear mixer in the presence of a surface active agent or an emulsifier which can stabilize the surface of the newly created smaller sized oil droplets (Fig. 2.2) (McClements, 2010; Sarkar, 2010). More recently, microfluidizers have also been used in the preparation of micro-emulsions that are also types of high-pressure homogeniser (Jafari et al., 2007). In a commercial environment, a limited number of technologies have been used to prepare finished emulsion-type food products. A colloid mill is generally used for the manufacture of mayonnaise or dressing type products. On the other hand,

cream and flavoured milk products are generally produced using a high-pressure homogeniser as stated above. Margarine and other water in oil type emulsified products are produced on votator lines (Appelqvist et al., 2007).

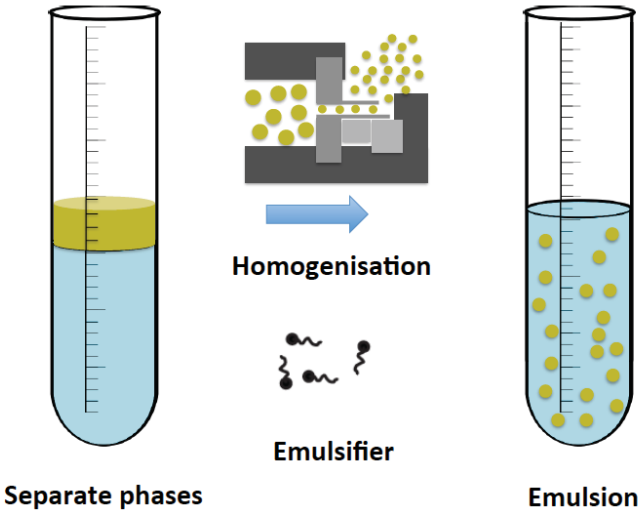


Figure 2.2 *Preparation of an emulsion from separate oil and water phase using an emulsifier and a high-pressure homogeniser (a schematic representation adapted from McClements, 2010).*

In any case, this procedure involves forcing the oil and water phase through a narrow orifice creating intense shear flow which results in breakage of bigger oil droplets into small sized droplets which are simultaneously covered by the surface active agent. These surfactants form a stable interfacial layer that provides stability to the droplets against disruptive interactions, such as coalescence (Dickinson, 2003, Sarkar, 2010). The concentration of the surface-active agent, the pH of the bulk

phase, viscosity, storage temperature, homogenisation pressures etc. are some of the factors that affect the stability of emulsions (Dickinson, 2003; Sarkar, 2010).

2.3 Emulsion stability

Stability after manufacture and packaging is the most important characteristic of food emulsions (Appelqvist et al., 2007). The term emulsion stability refers to the lack of change in emulsion characteristics over time. The time of observation can be from a few hours for lab scale samples up to a year or more for commercial products (McClements, 2005; Sarkar, 2010). Inherently, emulsions are unstable (Dickinson & Stainsby, 1982; Ye, 1999). The size and distribution of the oil droplets and their arrangement in space is always changing in emulsions over its storage life. Practically, if the changes in the emulsion characteristic happen rather slowly, it can be considered as stable (Dickinson et al., 1988; Ye, 1999). This is due to the free energy being positive as a result of a large surface area between the oil and water phase (Hunter, 1986; Sarkar, 2010). Therefore, in order to develop strategies for the stabilization of food emulsions, an understanding of the mechanisms of destabilization is essential (Appelqvist et al., 2007). The four main types of destabilizing mechanisms are briefly discussed in the following section (Fig. 2.3).

2.3.1 Creaming/sedimentation

The oil phase in most food emulsions has a lower density as compared to the aqueous phase making it susceptible to separation due to gravity. In the case of oil-in-water emulsion, if a concentrated layer of cream (mainly oil) is formed due to gravity, this type of destabilization of emulsion is referred to as creaming (Fig. 2.3). This mainly happens as a result of breakdown of the forces providing stability to emulsion. In case of emulsions with sufficient surface-active agents, the cream layer

can usually be redispersed and the dense droplets may settle at the bottom (Fig. 2.3). This is referred to as sedimentation (Appelqvist et al., 2007). In fairly dilute systems, the rate of creaming, ν_s, can be defined through Strokes' equation (Walstra, 1987):

$$\nu_s = \frac{2r^2(\rho_0 - \rho)g}{9\eta_0} \qquad (2.1)$$

Where g = acceleration due to gravity, r is the radius of the droplet, ρ is the density of the dispersed phase, ρ_0 is the density of the continuous phase and η_0 is the Newtonian shear viscosity of the continuous phase (Walstra, 1987; Appelqvist et al., 2007).

It is clear from the Strokes' equation that the rate of creaming may be reduced either by reducing the size of the oil droplets, increasing the viscosity of the continuous phase or by designing the emulsions with closer densities of the two phases. In order to reduce the extent of creaming of an emulsion system using the Strokes' equation in a quantitative manner, the emulsion system has to be very dilute. However, in concentrated and polydispersed emulsions the relationship between the rate of creaming and other parameters is not straightforward. The measurement of creaming can be simply made by visual observation. However, several non-invasive techniques, such as ultrasound, magnetic resonance imaging and conductivity have been used successfully to measure creaming (Dickinson, 1996; Appelqvist et al., 2007).

2.3.2 Flocculation

Flocculation refers to the association of emulsion droplets or reversible aggregation of emulsion droplets due to inter-droplet attractive and repulsive forces (Fig. 2.3). The interfacial layer on the droplets remains intact (Dickenson, 1998; Appelqvist et

al., 2007; Sarkar, 2010). In dilute emulsions, flocculation may increase the rate of creaming as the flocs rise more quickly compared to individual droplets. The same mechanism may not follow in denser emulsions as the flocs may form networks. Generally, two types of flocculation are noticed i.e. bridging flocculation and depletion flocculation (Dickinson, 2003; Singh & Ye, 2009). The type of flocculation that may prevail in an emulsion is dictated by the interfacial potential (Appelqvist et al., 2007).

2.3.2.1 Depletion flocculation

Generally, the origin of depletion flocculation is linked to the presence of excess non-adsorbed high molecular weight biopolymer in the continuous phase of the emulsion. The presence of these biopolymers promotes an osmotic pressure imbalance causing the droplets to floc together. Dickinson & Stainsby (1988) and Walstra (1993) explained depletion flocculation as arising from colloidal particles that are too close to each other that higher molecular weight biopolymers are excluded from that region of the continuous phase. As a result of the exclusion or depletion, solvent present between particles gets mixed with the bulk/continuous solvent, reducing the free energy and consequently causing inter-droplet attraction and flocculation (Fig. 2.4).

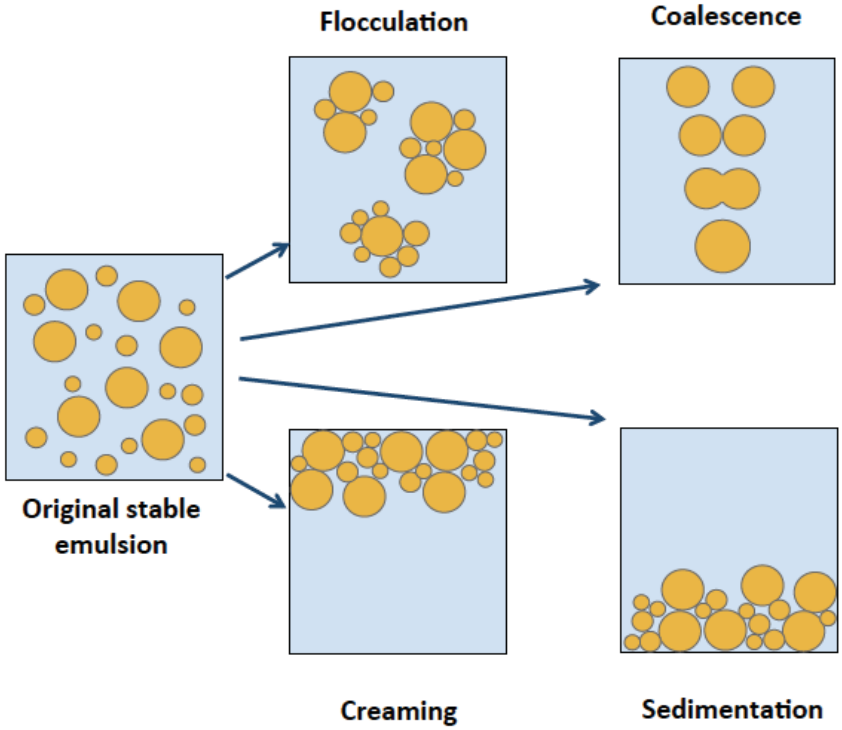


Figure 2.3 *Schematic representations of mechanisms for droplet instability in oil-in-water emulsions. Yellow represents the oil phase whereas blue represents the aqueous phase (adapted from McClements & Weiss, 2005).*

The bonds formed as a result of depletion flocculation are generally weak and reversible (Blijdenstein et al., 2003; Blijdenstein et al., 2004a). From a practical viewpoint a number of polysaccharides (guar gum, xanthan gum, gum arabic) and proteins (caseinates) can cause depletion flocculation when added in excessive amounts in emulsions (Dickinson & Golding, 1997; Tuinier et al., 1999; Tuinier et al., 2000; Blijdenstein et al., 2003; Blijdenstein et al., 2004b; McClements, 2010; Sarkar, 2010).

Dickinson and co-workers investigated the influence of sodium caseinate as the sole emulsifying agent in stabilizing oil-in-water emulsions with oil content of 35 or 45%

(%, vol). At a protein concentration of 2.0% (w/w), no creaming, flocculation or coalescence was observed in these emulsions for several weeks. However, at 3.0% (w/w) concentration of protein, serum separation was noticed as a result of depletion flocculation. Due to the presence of excess amount of caseinate in the bulk phase at 3.0% concentration, the critical concentration required to cause depletion flocculation was exceeded. If the same emulsion contains optimum amount of protein required to stabilize the oil droplet and saturate the interface of the droplet surface, kinetic stability of the emulsions is possible (Dickinson & Euston, 1991; Dickinson & Pawlowsky, 1997; Sarkar, 2010; Srinivasan et al., 1996).

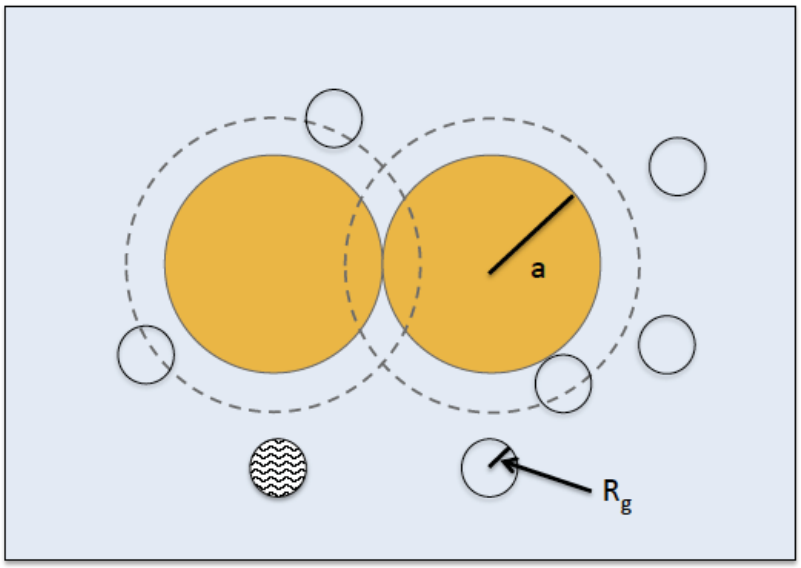


Figure 2.4 *A schematic representation of depletion flocculation where biopolymers with radius of gyration (R_g) are excluded/depleted from the solution between spherical particles (radius = a) (Walstra, 1993; Ye, 1999).*

The addition of low levels of polysaccharides, such as κ-carrageenan, can also cause depletion flocculation in emulsions where depletion flocculation is not observed at high concentrations such as in whey protein stabilized emulsions (Singh et al., 2003; Sarkar, 2010). This was thought to be caused by thermodynamic incompatibility between WPI (0.5-3.0%) and κ-carrageenan (0.025%) causing extensive flocculation (Singh et al., 2003; Sarkar, 2010).

2.3.2.2 Bridging flocculation

Bridging flocculation is simply the association of emulsions droplets through interfacial interaction. Bridging flocculation can occur via various mechanisms, which determine the strength of the interfacial interactions (Appelqvist et al., 2007). *Incomplete surface coverage bridging* occurs as a result of a significantly low concentration of a high molecular weight biopolymer in emulsion where bridges are formed between two or more emulsion droplets (Appelqvist et al., 2007; De Hek & Vrij, 1981; Dickinson, 1999; Tuinier & de Kruif, 1999; McClements, 2005; Singh & Ye, 2009, Sarkar, 2010). When present at the optimum concentration, higher molecular weight biopolymers provide sufficient protection against flocculation with the formation of thick/saturated interfacial layer. However, in case where the concentration of the biopolymer is at a lower level, attachment of the biopolymer may occur at two different droplets rather than attaching to just one. Following the same mechanism, several droplets may be bridged together resulting in a floc as shown in Figure 2.5 (Appelqvist et al., 2007; De Hek & Vrij, 1981; Dickinson, 1999; Tuinier & de Kruif, 1999; McClements, 2005; Singh & Ye, 2009, Sarkar, 2010). Emulsions homogenised at a very high pressure have also been reported to display flocculation through this particular mechanism (Appelqvist et al., 2007).

Bridging flocculation can also occur through the addition of counter ions in emulsion stabilized with ionic biopolymers. Also referred to as *electrostatic bridging*, addition of salts can enable bridges to be formed between droplets as seen in calcium ion bridging of emulsions droplets in casein stabilized emulsions at neutral pH. Divalent calcium ion binds to the negatively charged amino acids on the peptide chain of the casein proteins adsorbed at the oil droplet interface. This can cause caseins to be shared between different emulsion droplets (Sarkar, 2012).

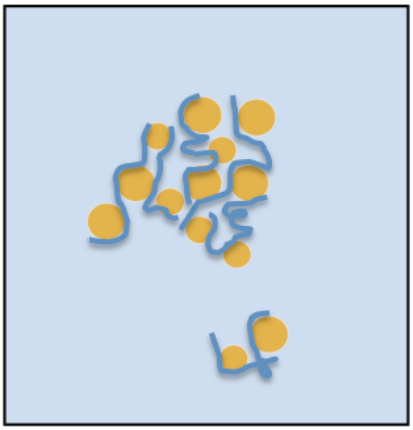


Figure 2.5 *A schematic representation of bridging flocculation in oil-in-water emulsion where oil droplets (yellow) are bridged together by biopolymers (blue line) (Adapted from Sarkar, 2010).*

Emulsions droplets can be *covalently bridged*, through the disulphide interchange reaction between proteins adsorbed at the interface of two emulsion droplets. This type of covalent cross-linking can be induced by applying heat treatment or static high pressure to the emulsion (Appelqvist et al., 2007; Galazka et al., 2000). Enzymatic covalent cross-linking can also be induced via microbial transglutaminase which catalyses cross-linking between glutamine and lysine amino acids. Emulsion

droplet in close proximity may consequently become cross-linked due to the reaction between the two amino acids where droplet separation is sufficiently low (Dickinson & Yamamoto, 1996; Appelqvist et al., 2007).

2.3.3 Coalescence

Coalescence, always irreversible, is an end result of the rupture of the interfacial film at the surface of the oil droplets (Singh & Ye, 2009). This fusion of two or more emulsion droplets to form one single droplet of greater volume occurs due to the thinning of the surface below a critical level. In instances where oil-in-water emulsions are produced using biopolymers, the interfacial layer is very stable against coalescence (van Aken et al., 2003; van Aken, 2004; Sarkar, 2010). However, thinning of the interfacial layer can occur as a result of any high stress imparting processing method, such as high shear mixing, enzymatic hydrolysis or dehydration (Agboola et al., 1998; Singh & Dalgliesh, 1998; Sarkar, 2010; Walstra, 1987).

An inverse relationship exists between viscosity of the continuous phase of the emulsion and the degree of coalescence. In addition, the rate of coalescence also depends on the concentration of the dispersed phase and viscoelastic behaviour of the absorbed layer. Increasing the interfacial viscoelasticity through polymer adsorption can increase emulsion stability against coalescence. Emulsions stabilized with small molecular weight surfactants do not possess the required viscoelasticity to prevent interfacial thinning during high shear processing conditions. During high shear conditions, stability against coalescence is provided in these systems through the Marangoni effect. Mobility of surfactant at the interface leads to thinning of the interfacial film which causes osmotic pressure differential between the film and

surrounding solvent. As a consequence, water is drawn in at the location of these gaps, which prevent further thinning.

Generally, coalescence follows first order kinetics in emulsion systems, which can be expressed as (Walstra, 1987; Sarkar, 2010):

$$\frac{N_t}{N_o} = e^{-KcT} \qquad (2.2)$$

Where, N_t is the number concentration of emulsion droplets at time t; N_o is the initial number concentration of freshly homogenized emulsions droplets (time zero); K_c is the coalescence rate constant in time t (Darling, 1987; Sarkar, 2010). The rate of coalescence can be obtained from the slope of the plot between (N_t/N_o) versus time (Walstra, 1987).

2.3.4 Ostwald ripening

The growth of a larger emulsion droplet at the expense of a smaller one is referred to as Ostwald ripening (Appelqvist et al., 2007). The solubility of a material making up an emulsion particle would be greater for a smaller particle as compared to a larger particle (Fig. 2.6), according to Kelvin equation (2.3) as below:

$$\frac{s(r)}{s_\infty} = \exp\left(\frac{x'}{r}\right)$$
$$x' = \frac{2\gamma V_D}{RT} \qquad (2.3)$$

Where s is solubility, γ is the interfacial tension, V_D is the molar volume of the material in disperse phase, R is the universal gas constant and T is temperature (Walstra, 2003).

The driving force behind Ostwald ripening is the difference in chemical potential due to the difference in radius, where the molecules move through diffusion (Walstra, 2003). Due to the negligible solubility of triglycerides in water, Ostwald ripening is not observed in most oil-in-water food emulsions. However, some essential oils, such as from citrus fruits, contain of considerable amount of various terpenes which are soluble in water. Emulsions made for applications in foods as flavouring ingredients can show Ostwald ripening (Walstra, 2003).

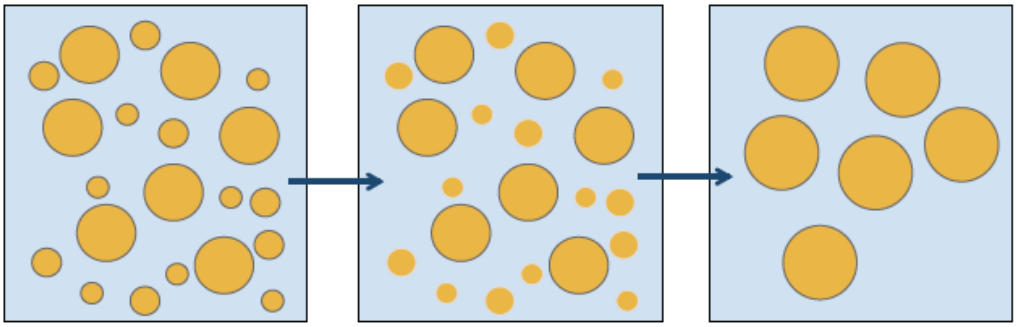


Figure 2.6 *A schematic representation of Ostwald ripening (adapted from Taylor, 1998). Oil droplets are represented in yellow and the continuous phase (water) is represented by blue.*

2.4 Milk protein-stabilized emulsions

Milk proteins, being amphiphilic in nature, are known to be excellent emulsifiers and stabilizers. They exhibit good surface-active properties by reducing the surface tension when adsorbed at the oil-water interface (Morr, 1982; Mulvihill & Fox, 1989; Sarkar, 2010). There are two main classes of milk protein; caseins, which are sensitive to change in pH, and whey proteins, which are sensitive to heat (Fox, 2001;

Singh, 2011). Both caseins and whey proteins display different molecular and physico-chemical properties (Fox, 2001). Caseins are made up of four distinct proteins classified into α_{s1}-, α_{s2}-, β- and κ–caseins that are all phosphoproteins (Fox, 2009; Singh, 2011). All of the caseins are high in proline, which inhibits the formation of a secondary structure (Fox, 2001). This lack in secondary structure renders them stable against heat denaturation (Fox, 2001). They are also unable to sufficiently remove their hydrophobic groups from contact with water. This leads to self-association of the caseins (Singh, 2011). Majority of caseins exist as colloidal particles called caseins micelles in milk with an average diameter of ~150nm. The casein micelles also contain small amounts of calcium, phosphate, citrate and magnesium (Horne, 2003; Singh, 2011). The casein micelle has been characterised by a 'hairy' appearance, which comes from κ–casein found protruding from the surface of the micelles. These charged hair prevent the interactions between casein micelles (Fox, 2001, Singh, 2011).

Whey proteins, mainly fractionated into β-lactoglobulin, bovine serum albumin, α-lactalbumin and immunoglobulin, possess a high degree of secondary, tertiary and in most cases quaternary structures (Singh, 2011; Fox, 2001). Whey proteins are typical globular proteins and are denatured on heating e.g. completely at 90°C for 10 mins. A common feature of all whey proteins is intramolecular disulphide bonds that stabilize their structure (Fox, 2001). β-lactoglobulin contains sulfhydryl groups buried inside the molecule. Upon heat-treatment these sulfhydryl groups become exposed and active and can undergo sulfhydryl-disulphide interactions with other sulfhydryl groups of another β-lactoglobulin or other proteins (primarily κ–casein) above a heating temperature of 75°C x 15s (Fox, 2001). From a nutritional and physiological viewpoint, the proteins in milk are, arguably, the most important

constituent (Fox, 2001). For this reason milk proteins are used in a wide variety of prepared foods (Singh, 2011). Various protein products are manufactured by the dairy industry such as caseins and caseinates, whey-protein concentrates and isolates, milk protein fractions (Singh 2011). The applications of these milk protein products are found in meat products, dairy products, beverages, baked products and infant foods (Singh, 2011). Even though, milk proteins have several important functional properties including foaming, binding, whipping, gelation and emulsification, only emulsion stabilizing properties have been considered for this review.

During homogenisation, milk proteins become rapidly adsorbed on the newly formed oil droplets (Singh & Ye, 2009). The amount of protein that is present on the surface of oil droplets is expressed in milligrams of protein per unit area of the dispersed phase. The protein load in emulsions can be affected by the type of protein, the concentration of protein present at the time of emulsification, pH, ionic strength, oil to protein ratio, temperature and the presence of calcium ions (Dickinson & Stainsby, 1988; Walstra, 1993; Singh & Ye, 2009).

The main factors that determine the effectiveness of the milk proteins in lowering the surface tension are the type and number of contacts the protein makes at the oil-water interface. As caseins are more flexible, a substantially higher proportion of non-polar residues are in contact with the interface, which makes them more effective in reducing the surface tension as compared to whey proteins (Dickinson & McClements, 1995; Singh & Ye, 2009). The sequence of surface activity of milk proteins beginning with the most surface active is β-casein > monodispersed casein micelles > serum albumin > α-lactablumin> α_s-caseins = κ–casein > β-lactoglobulin (Mulvihill & Fox, 1989; Singh & Ye 2009; Sarkar, 2010).

Proteins rearrange and unfold once adsorbed at the oil droplet interface. The more flexible the protein, the more rapid the unfolding at the interface. At lower concentrations in emulsion, caseins are stretched to their maximum extent at a protein load of 1 mg/m^2. On the other hand, when excess protein is present, a more compact confirmation is adopted at the interface with a protein load of up to coverage 3mg/m^2 approximating monolayer coverage. Whey proteins also show a change in confirmation by unfolding at the surface but this unfolded confirmation has been reported to lie somewhere intermediate between native-like and fully denatured (Dickinson, 1998). It has been suggested that a protein load of < 1mg/m^2 is indicative of fully unfolded proteins at the oil-water Interface. A protein load of 1-3 mg/m^2 suggests that a protein monolayer may be present. In case aggregate proteins or protein multilayer are present, a high protein load is observed (>5 mg/m^2) (Phillips, 1981; Hunt & Dalgleigh, 1994; Singh & Ye, 2009).

2.4.1 Caseins as adsorbed biopolymers

Studies done on purified milk protein systems show that caseins produce an entangled monolayer once absorbed on the surface of the oil droplets. Casein monomers are regarded as complex linear copolymers having flexible chains with some segments in direct contact with the oil droplet surface (trains) and other segments protruding into the aqueous phase (loops and tails) (Dickinson, 1998; Singh & Ye, 2009). α_{s1}-Casein and β-casein make up almost 75% of the total casein present in milk and are expected to provide similar emulsification based on their amino acid profile (Swaisgood, 1982; Sarkar, 2010). However, based on experimental analysis, β- casein exhibits a higher rate of lowering of interfacial tension as compared to α_{s1}- casein (Dickinson, 1998; Sarkar, 2010). β-Casein has

been shown to bind strongly with the oil phase using its hydrophobic region, while the hydrophilic region protrudes into the water phase (Dalgliesh, 1996; Dickinson, 1999; Sarkar, 2010). This has been referred to as the 'tail-like' anchoring. On the other hand, α_{s1}- casein binds to the oil surface via peptides towards the middle of the sequence also referred to as 'loop-like' anchoring as shown in Figure 2.7. In addition, β-casein has shown competitive adsorption at the oil-water interface as compared with α_{s1}- casein in purified milk protein systems. β-Casein also appeared to displace α_{s1}- casein from the droplet surface because of its high relative surface activity (Dickinson & Stainsby, 1998; Dickinson et al., 1988, Dalgleish, 1993; Dalgleish, 1996; Sarkar, 2010).

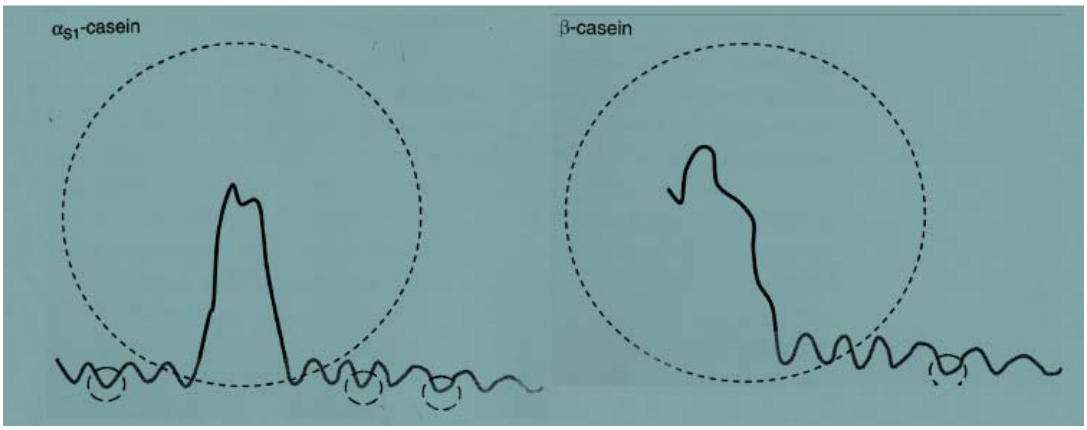


Figure 2.7 *Schematic of α_{s1}- casein and β-casein illustrating the 'loop-like' anchoring of α_{s1}- casein and the 'tail-like' anchoring of β-casein onto a hydrophobic interface. The larger circles depict the range of electrostatic repulsions arising from the negative charge centres and the smaller circles depict the hydrophobic regions (Singh & Ye, 2009)*

However, the milk protein products used in food industry usually contain complex mixtures of proteins with varying degree of aggregation. The nature of the adsorbed layer in emulsions made using these protein products is still not clear (Singh & Ye, 2009). Caseinates are produced from skim milk by lowering the pH to 4.6, either by addition of acid or microbial cultures to precipitate the caseins. The precipitated caseins are then re-solubilized with alkaline salts of sodium, potassium or calcium at neutral pH followed by spray drying into powders (Mulvihill, 1989; Sarkar, 2010). The resulting products are referred to as sodium, potassium or calcium caseinate. The most commonly used caseinates are sodium caseinate and calcium caseinate (Singh & Ye, 2009).

Sodium caseinate, which consists of α_{s1}-, α_{s2}-, β-, and κ–casein, show excellent emulsifying ability along with the capability of producing stable emulsion at the relatively low protein-oil ratio (~1:60). The protein load has been shown to plateau at around 2-3 mg/m^2 with the increase in protein concentration in emulsions made using sodium caseinate (Singh, 2005; Singh & Ye, 2009). On the other hand, about 25% of the total casein in calcium caseinate is in an aggregated form (Srinivasan et al., 1999). Emulsions made with calcium caseinate require high concentration of protein to produce a stable emulsion. Also, larger droplets are formed when similar concentration of protein is used to prepare emulsions containing calcium caseinate as compared with sodium caseinate (Euston & Hirst, 1999; Singh & Ye, 2009). Depending on the protein concentration, a protein load of 5-20 mg/ m^2 has been reported for emulsion made using calcium caseinate (Euston & Hirst, 1999; Singh & Ye, 2009). This has been suggested to be due to a lower spreading capability on the oil interface as the aggregates are held together by calcium bonds and/or colloidal calcium phosphate. The higher confirmation stability is unlikely to be affected

during the emulsification process contributing to the reduced emulsifying ability (Euston & Hirst, 1999, Srinivasan et al., 1999; Singh & Ye, 2009).

In emulsion stabilized with sodium caseinate, no competitive adsorption has been observed. This was in contrast with the findings in purified casein systems. Studies have shown that competitive adsorption of caseins in sodium caseinate stabilized emulsions appears to be related to the protein-oil ratio (Srinivasan et al., 1999; Singh & Ye, 2009). At lower protein concentrations, β-casein is preferentially adsorbed at the droplet surface. However, when sodium caseinate concentration is in excess of the amount required for full droplet coverage, α_{s1}- casein is adsorbed in preference to one of the caseins. This loss in competitive adsorption of β-casein has been suggested to be due to self-association of β-casein or association with other caseins (due to a higher surface activity) at a higher concentration of caseins in solution (Lucey Iet al., 2000; Singh & Ye, 2009). A wide range of information is available on the surface activity of individual caseins but the in depth understanding of the characteristics of caseins in aggregated and complexed form is absent (Singh & Ye, 2009).

2.4.2 Whey proteins as adsorbed biopolymers

About 70 to 80% of the total whey proteins present in milk comprise of β-lactoglobulin and α-lactalbumin. Although less surface active than β-casein, both β-lactoglobulin and α-lactalbumin adsorb to the oil-water interface to form stable emulsions (Hunt & Dalgleish, 1994). β-Lactoglobulin, the main whey protein, mostly exists as a non-covalently linked dimer under ambient conditions with an approximate molecular weight of ~36 kDa at pH 7 (McKenzie & Sawyer, 1967; Ziegler & Foegeding, 1990; Sarkar, 2010). When used in emulsions, β-lactoglobulin

partially unfolds on the oil droplet interface creating continuous film through β-pleated sheets interactions, exposing reactive sulfhydryl groups. Once exposed these sulfhydryl groups can self-aggregate via sulfhydryl-disulphide interchange reaction (Dickinson & Matsumura, 1991; McClements et al., 1993; Lefevre & Subirade, 2003; Sarkar, 2010). α-Lactalbumin, on the other hand, has no free thiol group but has four intra-chain disulphide bonds (Swaisgood, 1982; Brew & Grobler, 1992; Sarkar, 2010). Even though, the denaturation temperature of α-lactalbumin is lower than β-lactoglobulin, it doesn't self-aggregate due to absence of the free thiol group (Dalgleish et al., 1997). However, in a binary mixture of β-lactoglobulin and α-lactalbumin, sulfhydryl-disulphide interchange does occur.

Some competitive adsorption has been reported for binary mixtures of β-lactoglobulin and α-lactalbumin. However, displacement of one protein by the other has not been observed. The protein dominating the interface at the time of homogenisation remains at the interface thereafter (Singh & Ye, 2009). The two main whey protein products commonly used in the food industry are whey protein concentrate (WPC) and whey protein isolate (WPI). The protein levels obtained after processing are around 80 to 95% (Mulvihill & Ennis, 2003; Singh, 2005; Sarkar, 2010). As mentioned earlier, whey proteins tend to denature and aggregate with the application of heat above 70°C. During the manufacture of WPI and WPC, some denaturation of the whey protein has been reported to occur which has an impact on its functionality. No preferential adsorption has been observed between β-lactoglobulin and α-lactalbumin in emulsions that are prepared using WPC or WPI (Euston et al., 1996; Singh & Ye, 2009).

2.5 Factors affecting the stability of milk-protein stabilized emulsions

2.5.1 Effect of homogenisation pressure

The effectiveness of milk-proteins as emulsifiers is impacted by homogenisation pressure (Tornberg & Hermansson, 1977; Murphy & Fox, 1991; Ye, 1999). Up to a certain limit, greater energy input in the form of homogenisation pressure would result in more stable emulsions. With the increase in homogenisation pressure, the average droplet diameter reduces and the specific surface area increases. This results in an increase in emulsion stability. However, in some cases prolonged homogenisation results in destabilisation of the emulsion due to whey protein denaturation and aggregation (Tornberg & Hermansson, 1977; Haque & Kinsella, 1987; Ye, 1999). Several studies have reported a decrease in the average droplet diameter values with the increase in homogenisation pressures (Srinivasan et al., 1996; Jafari et al., 2008; Ye, 1999) in emulsions made using milk proteins. At the same time, the protein load was shown to decrease, which was due to increased spreading, and rearrangement of the proteins at the surface of the oil droplets (Mulvihill & Murphy, 1991; Ye, 1999).

2.5.2 Effect of protein and oil concentration

With the increase in protein concentration, the average droplet diameter decreases with concomitant increase in protein load. However, beyond a certain protein concentration, the average droplet diameter does not decrease any further. At high protein concentration, the interfacial layer tends to pack more efficiently which is the reason behind a higher protein load (Hunt & Dalgleish, 1994; Fang & Dalgleish, 1993; Ye, 1999). The increase in the concentration of oil has the opposite effect to

increase in protein concentration. With more oil present in the system, the interfacial layer becomes thinner with greater spreading of the protein due to an increase in the specific surface area (Srinivasan et al., 1996; Ye, 1999).

2.5.3 Effect of pH

In caseinate stabilized emulsions, with decrease in pH from 7 to 6, enhanced flocculation has been observed which was due to the decrease in electrostatic repulsion between adsorbed proteins leading to bridging flocculation (Tornberg & Ediriweera, 1988). In emulsions stabilized with β-casein, the protein load was observed to increase with the decrease in pH towards the isoelectric point. This was suggested to be due to the association of β-casein molecules at the interface as well as in solution (Dickinson & McClements, 1995; Ye, 1999). Stable emulsions can be prepared with both caseinates as well as whey proteins under acidic conditions (pH 3) as well as at pH 7 (Hunt & Dalgleish, 1994; Ye, 1999). The change in pH has also been reported to affect the composition of the interface as seen with the preferential adsorption of α-lactalbumin at lower pH. However, the impact of pH on emulsions stabilized with milk proteins depends largely on the concentration of protein in the emulsion (Hunt & Dalgleish, 1994; Ye, 1999).

2.6 Heat-induced changes in emulsions stabilized with milk proteins

In order to improve the quality and extend the shelf life of milk and milk products, thermal processing is essential and is a commonly used unit operation in the dairy industry. Thermal processing is also essential to reduce the microbial load of milk and to minimize the risk of food poisoning (McKinnon et al., 2009; Raikos, 2010). However, in some cases heat treatment is also employed to manipulate the functional

properties of milk proteins to improve the organoleptic properties of certain dairy formulations (del Angel & Dalgleish, 2006; Raikos, 2010). The modification of functional properties of milk as a result of the heat treatment have been extensively documented in literature. Depending on the processing conditions of milk or systems containing proteins, several mechanisms have been proposed to justify the findings (Lucey et al., 1999; Morr, 1985; Singh & Newstead, 1992; Raikos, 2010). In the following sections, we focus on the impact of heat treatment on the formation and stability of emulsions prepared using milk proteins.

2.6.1 Heat treatment of milk proteins

At elevated temperatures, whey proteins denature which is accompanied by unfolding and exposure of its hydrophobic groups. β-Lactoglobulin is known to self-associate with the increase in heating time and temperature (Jang & Swaisgood, 1990; Raikos, 2010). α-Lactalbumin also denatures at higher heat treatment temperatures however aggregates are only formed with β-lactoglobulin (Fox, 1992; Raikos, 2010). It has been suggested that the type of aggregate formed from β-lactoglobulin as a result of heat treatment are dependent on the pH of the system. Aggregates are thought to be branched at neutral pH; spherical, close to the isoelectric point and fibrillar at pH 2 (Nicolai et al., 2011). Nicolai and co-workers described the aggregation of β-lactoglobulin at pH >5.7 as shown in Figure 2.8.

Upon denaturation, small sized oligomers (mainly dimers and trimers) start to form as a result of convent linking (molecular bonds formed as a result of electron sharing) via disulphide bonds. With the progression of oligomer formation above a critical concentration, primary aggregates are formed. The shape of the primary aggregates depends on the pH of the solution. These aggregates can be curved or stranded at neutral pH whereas at pH 5.8, they are observed to be spherical in shape.

With the increase in concentration of protein these primary aggregates grow into larger sized polydispersed aggregates. Above a critical concentration of protein in the system, a gel network is formed where loss of flow may be observed on tilting the container (Nicolai et al., 2011).

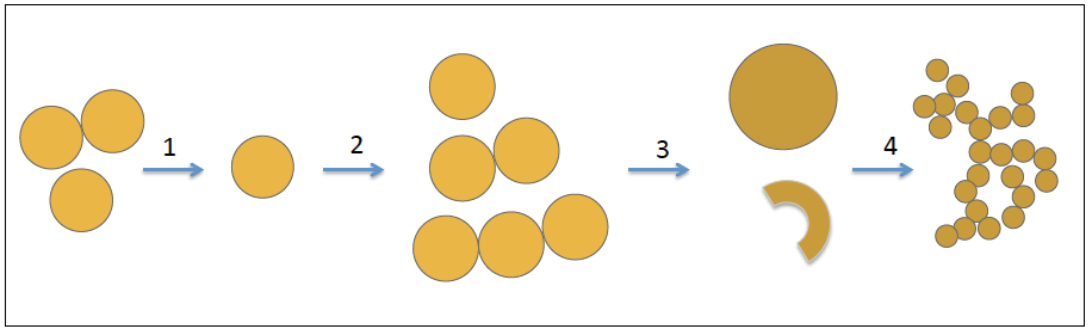


Figure 2.8 *Schematic representation of the aggregation process in β-lactoglobulin systems at pH > 5.7. The process starts with the dissociation of dimers into monomers as a result of denaturation (step 1). Larger oligomers start to form with the progression of heating (step 2). Above a critical concentration of protein, larger primary aggregates are formed which may vary in shape and size (step 3). With further progression of the process, self-similar aggregates may be formed, which may precipitate or gel formation may occur (step 4). The scale in the schematic changes at step 3 and 4 (Adapted from Nicolai et al., 2011).*

Upon heat treatment of β-lactoglobulin, the equilibrium shifts due to which the proteins change to the monomeric state as opposed to normal dimers in native state. In systems where both caseins and whey proteins are present (milk), following denaturation, complexes between β-lactoglobulin and κ–casein are formed (Oldfield et al., 2000; Raikos, 2010). In milk, these β-lactoglobulin and κ–casein complexes

could be present on the surface of the casein micelle as well in the serum phase of milk. Whey protein only aggregates are also formed but reports suggest that the β-lactoglobulin and κ–casein preferentially bind via hydrophobic/di-sulphide interchange. This also plays a role in the reduction of the size of whey protein only aggregates (O'Kennedy & Mounsey, 2006; Raikos, 2010). Factors impacting the denaturation and aggregation of whey proteins are heating time/temperature, protein concentration and pH of the system (Anema & Li, 2003; Raikos, 2010). On heating of milk whey protein aggregates are found on the surface of the casein micelle at pH <6.6. On the other hand, at pH of higher than 6.6 whey protein aggregates were present in the serum phase (Vasbinder & de Kruif, 2003; Raikos, 2010; Singh & Fox, 1995).

2.6.2 Impact of heat treatment in milk protein-stabilized emulsions

Milk protein functionality changes upon heat treatment. However, the change is different for caseins than whey proteins. Heat treatment of sodium caseinate at 50-100°C resulted in increase in its emulsifying abilities. This was suggested to be due to heat induced exposure of its hidden hydrophobic domains, which were previously hidden at the protein back bone (Jahaniaval et al., 2000; Raikos, 2010). In contrast, whey proteins showed a loss in emulsifying ability with heat treatment between 60-90°C for up to 1000s. The most likely cause of the loss in functionality was due to formation of large aggregates, which were unable to cover the surface of the oil droplets efficiently (Millqvist-Fureby et al., 2001; Raikos, 2010). Millqvist-Fureby and co-workers reported an increase in the average droplet diameter with the increase in time/temperature of heating in emulsion made using heat-treated whey protein solutions. However, the impact of heat treatment in protein solution containing mixtures of whey protein and caseins requires further exploration.

The impact of heating on the particle size of milk protein stabilized emulsions has been well documented (McSweeney et al., 2004; Raikos, 2010). Upon heat treatment of the emulsion (pH 6.8) at 140°C for 80 sec, a marked shift in the size distribution was noticed as the average droplet diameter shifted from < 1μm to 1-10μm. This shift in the particle size distribution was suggested to be a result of whey protein denaturation and aggregation between whey protein molecules present in the bulk phase of the emulsion and the whey protein adsorbed at the oil droplet interface (McSweeney et al., 2004; Raikos, 2010). Euston and co-workers (2000) referred to the denatured whey proteins present in the bulk phase of the emulsion as "glue" which promote the aggregation of emulsion droplets by interacting with the whey proteins adsorbed to the oil droplet interface and entangling emulsion droplets in large aggregates. Other studies suggested the increase in the particle size of the emulsion upon heat treatment due to droplet flocculation as a result of disulphide-mediated polymerization between whey proteins (Monahan et al., 1996; Raikos, 2010).

A different trend in particle size was reported by Sliwinski and co-workers (2003), where an increase in the droplet size was observed initially when the emulsion was heated between 65-80°C due to droplet flocculation. With an increase in the heating temperature to 90°C a decrease in the particle size was noticed. This behaviour of emulsion system was attributed to the extent of denaturation of whey proteins at the oil droplet interface. At lower temperatures of 65-80°C, an increase of surface hydrophobicity may promote droplet flocculation as whey protein are only partially unfolded. However, with the increase in heating temperatures the proteins become fully unfolded leading to effective rearrangement of the non-polar amino acids toward the oil phase. This would reduce the tendency of whey proteins on the

surface to aggregate and possibly form compact layers at the droplet interface (Manohan et al., 1996; Raikos, 2010). An increase in the protein load has also been observed in heat-treated emulsions made using whey proteins (Sliwinski et al., 2003; Raikos, 2010; Ye, 2010).

While a number of studies have shown an increase in the particle size as a result of heating due to flocculation, some studies have shown no change in the particle size due to heat treatment of whey protein stabilized emulsions (Hunt & Dalgleish, 1996; Ye, 2010). No change in the particle size was observed when an emulsion prepared using 2.0% (wt. %) whey protein isolate was heated at 90°C (30 min) or 121°C (10 min) at pH 7.0 and 3.0 (Hunt & Dalgleish, 1996). The difference in these findings could be possibly due to compositional differences but no suggestions were made by the authors.

As noticed in whey protein stabilized emulsions, emulsions stabilized with β-lactoglobulin alone also showed an increase in the particle size due to flocculation when heated at 85°C for various heating times (30 min – 48hr). However, with the addition of a very small amount of sodium caseinate into the emulsion, no change in the particle size was observed at the similar heating time temperature profile (Dickinson and Parkinson, 2004). It was suggested by the authors that steric stabilization, as a result of the presence of caseins and their long dangling tails, prevented the flocculation of emulsion droplets as shown in Figure 2.9 (Dickinson & Parkinson, 2004).

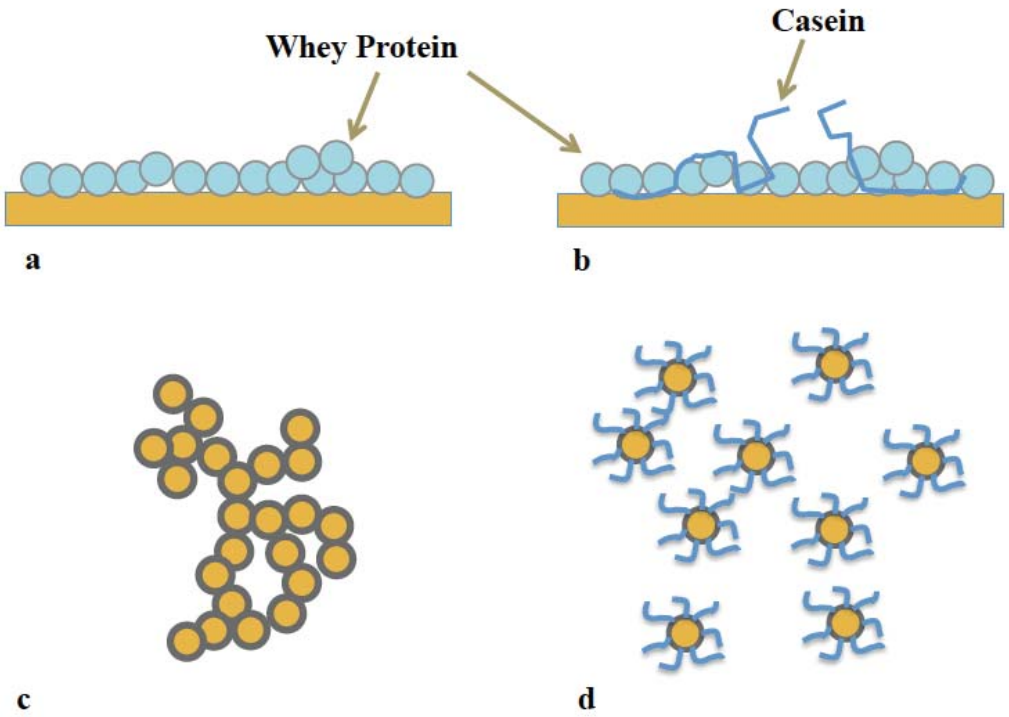


Figure 2.9 *Schematic representing the effect of heating on emulsions containing whey proteins alone and in emulsion containing both whey proteins and caseins. Whey protein adsorbed at the oil interface (a), whey proteins and caseins co-adsorbed at the oil interface with tails of the casein molecules protruding/dangling out of the interface (b), flocculation of emulsion droplets in whey protein stabilized emulsions due to heat treatment above 75°C (c) and sterically stabilized emulsion droplet due to caseins tails (d) (Adapted from Dickinson & Parkinson, 2004).*

In the last three to four decades, milk protein emulsions have been subjected to extensive research. As described in the previous sections, the most common manifestations of emulsions instability such as creaming, flocculation etc., which render such systems as unacceptable, have been thoroughly examined (Robbins et al., 2002; Vega & Roos, 2006).

In a commercial environment, a product with high moisture content such as an emulsion would pose challenges such as low shelf life, higher packing cost and high shipping and handling costs. An alternative means of increasing the shelf life of emulsions and also making them easier to transport and handle is by transforming them into powders (Vega & Roos, 2006). One of the oldest drying processes used in the food industry is spray drying. Due to the lower cost of processing and widely available equipment, spray drying has been used for decades for drying of food products, such as coffee, milk, eggs, flavours, creamers etc. A lot of literature is available where characteristic of whole milk powder and skim milk powder have been looked at (Vega & Roos, 2006). The following section excludes discussion regarding these products but rather focuses on dehydration of milk-protein stabilized emulsions. Firstly, the focus is on the theoretical aspects of the spray drying process followed by influence of composition and processing conditions. The application of spray drying and the industrial approaches used in microencapsulation of long chain polyunsaturated fatty acids have also been covered.

2.7 Spray drying: technical summary

Spray drying is a commonly used unit operation by which water can be removed from a liquid product to convert it into powder. The liquid product in atomised in a current of hot gas which in most cases is air, however nitrogen can be also be used (Fig. 2.10). The liquid product can be a solution, an emulsion or even a suspension. The size of the resulting particles varies from very fine (10-50 μm) to coarse particles (2-3 mm). By removing water from the product, chemical stability as well as microbiological stability can be obtained. The desired level of moisture of a powdered product lies between 1-3% (Vega & Roos, 2006; Gharsallaoui et al., 2007).

A dairy based powder is not only specified by its nutritional composition but also by its microbiological specifications as well as physical properties. Some of the physical properties used in specification of dairy-based powders are bulk density, flowability, wettability, dispersibility, insolubility index, white fleck number, free fat and appearance which are basic quality parameters with well defined methods and upper and lower specs depending on the product. The physical properties are hugely dependent on the processing conditions used during drying (Schuck, 2002). Spray drying process can be broken down into four sub processes, which have been presented in the section that follows. To achieve desired characteristic in the finished product knowledge of these sub-processes is critical (Vega & Roos, 2006; Gharsallaoui et al., 2007).

2.7.1 Atomisation

The atomisation of a liquid product into tiny droplets is most commonly achieved by using pressure (using high pressure pumps to feed emulsions) or centrifugal force. Commercially used nozzles include pneumatic atomisers, pressure nozzles, two fluid nozzles and sonic nozzles. The objective of atomisation is to maximise the surface area of the liquid product for optimum heat transfer to occur. By far, the most critical factor while selecting an atomiser type is the feed/product viscosity and the desired qualities of the finished product. The viscosity of the product also dictates the running conditions as well as the particle size of the droplets (Gharsallaoui et al., 2007).

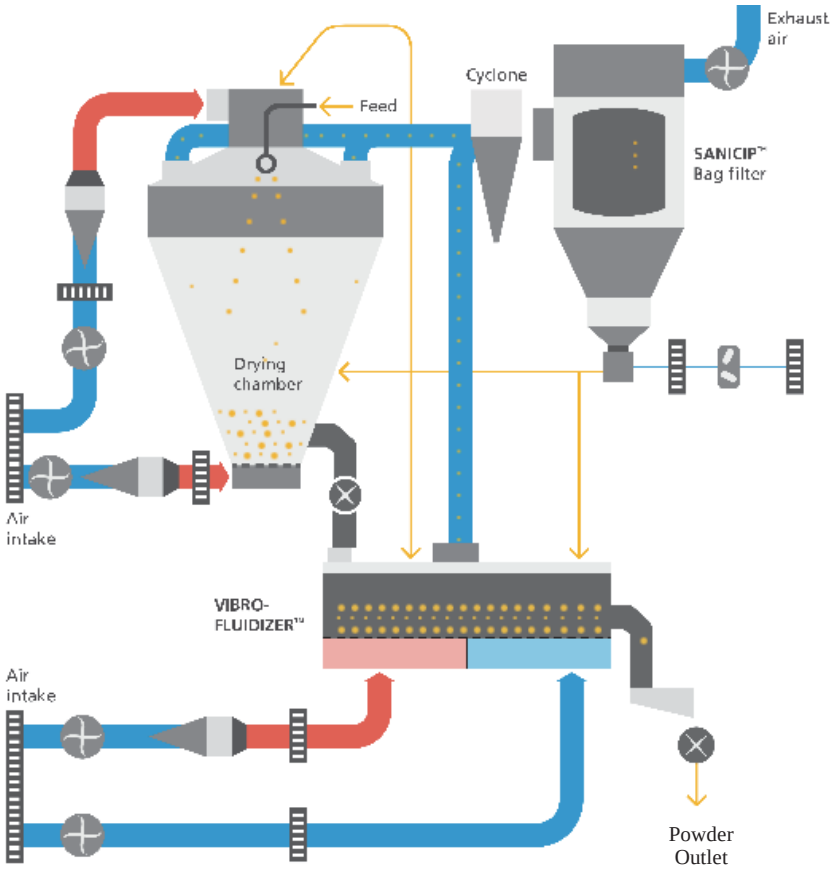


Figure 2.10 *A Schematic representation of the spray drying process (multi-stage dryer) commonly used in the manufacture of dairy products (Spray Dryer MSD™ Spray Dryer, 2015).*

2.7.2 Droplet-hot air contact

The contact of hot air and the droplet takes place inside the drying chamber. This is the step when the drying of the droplets is initiated. Depending on where inside the dryer the position of the hot air inlet is, the dryers can either be of co-current or counter current type. In counter-current type of dryer, the hot air and the feed/liquid product enter the drying chamber from the same direction leading to rapid dehydration. Typically, the inlet temperatures are approx. 150-220°C whereas the

droplets reach temperatures around 50-80°C. The product is subjected to higher temperatures in counter-current dryer setting. Although, counter current is comparatively more economical to run, it has limited use for thermo-sensitive products.

2.7.3 Water evaporation

As soon as the atomised droplets of the liquid product come in contact with the hot air, temperature and vapour pressure balances are established. Heat transfer takes place from the hot air to the liquid product and water transfer takes place in the opposite direction. Based on fundamentals of drying, the dehydration of a liquid product into powder can be divided into three steps. Firstly, the rise in temperature of the liquid droplet occurs as a result of hot air-liquid contact. Following this, temperature settles at a constant value while surface water evaporation proceeds at a constant rate. The diffusion of water from the core of the drying droplet to the surface of the droplet is also considered as constant and equal to the surface water evaporation. Finally, when the majority of the water has evaporated and a dry crust on the surface of the droplet is formed from which the evaporation rate decreases markedly resulting in the increase of the droplet temperature. Theoretically, drying is complete once the outlet air temperature and the particle temperature have reached equilibrium (Gharsallaoui et al., 2007). According to Birchal et al. (2006), the drying process can be divided into two periods, namely the constant rate period and falling rate period (Fig. 2.11). The constant period is when the evaporation of water proceeds at a constant rate. This is also accompanied by a drop in the product temperature. The second period is the falling rate period where the evaporation rate decreases significantly and crust formation starts to occur (Kim et al., 2008).

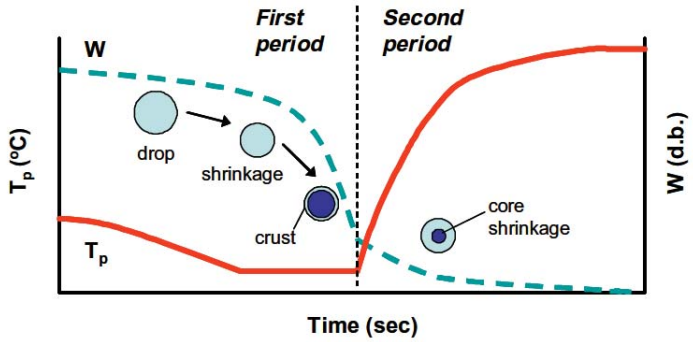


Figure 2.11 *Representation of the drying mechanism of a single milk particle as a function of residence time in the drying chamber. T_p represents the temperature of the particle and W represents water content of particle (Birchal et al., 2006; Kim et al., 2008).*

Depending on the drying conditions, the duration of these steps can be different. For instance, if the drying temperature is very high, the crust formation will take place very quickly because of a high water evaporation rate early in the process. Inherently, the process moves from liquid to solid particle very rapidly due to the large surface to volume ratio of the atomised droplets. Generally, total drying time is around 15-30 sec, which also represents the passage of the sprayed particle through the drying chamber (Gharsallaoui et al., 2007).

2.7.4 Powder separation

The majority of particles are collected at the base of the dryer at the internal fluid bed. The fluid bed bubbles hot air through the powder to aid further drying of the powder particles. However, finer particles may not settle on the bed and are generally collected and separated through the cyclones (Fig. 2.8). The cyclone-collected fines may be fed back into the dryer for agglomeration. External fluid beds are often incorporated into the design as a final dying stage then a cooling stage.

Fine particles may be elutriated off the external fluid bed and may also be caught in cyclones for use in agglomeration. The integration of fluid beds both inside and outside the dryer help control the particle size and bulk density of powders. Finally, the exhaust air from the cyclones is usually drawn through a bag house, which contains bag filters, which remove the finest of particles from the exhaust air stream. The powder particles that get filtered out through the bag filters may be disposed of due to the risk of contaminated by fibres from the bag material. The design and development of dryers has been based traditionally on trial and error and pilot plant studies. However, with the development of computational fluid dynamics (CFD) and the ability to measure size of particles, dryer design can be designed to the product requirements. CFD has also been used to model fouling on the dryer cone and the stickiness of particles (Gharsallaoui et al., 2007).

2.8 Properties of dried emulsions

The physical and chemical properties, such as flow, ease of reconstitution, storage stability of dairy powders and related systems have been subjected to extensive research recently (Buma, 1971, Bronlund & Paterson, 2004; Fitzpatrick et al., 2004; Vega et al., 2005, Kim et al., 2002). Some of most important properties of dehydrated emulsions have been discussed in the following sections.

2.8.1 Surface composition

The external surface of the powder particle after spray drying dictates the behaviour of the bulk powder with respect to physical properties such as flowability, wettability, stability to caking as well as chemical properties such as oxidative stability. The main component present on the powder particle surface that may alter the above-mentioned properties is “free” fat. The definition of surface free fat is very

broad as it is measured using an extraction method using organic solvents. However, the methods for measuring surface free fat vary as parameters like contact time, shaking vs. no shaking and temperature are not aligned. In addition, surface free fat may be extracted from the interior of the particle via cracks and holes in the powder particle as shown in Figure 2.12 (Buma, 1971; Bucchheim, 1982; Faldt et al, 1993; Vega & Roos, 2006). However, it is quite clear that surface free fat is strongly correlated with oxidative stability of fat-filled powders (Granelli et al., 1996; Vega & Roos, 2006).

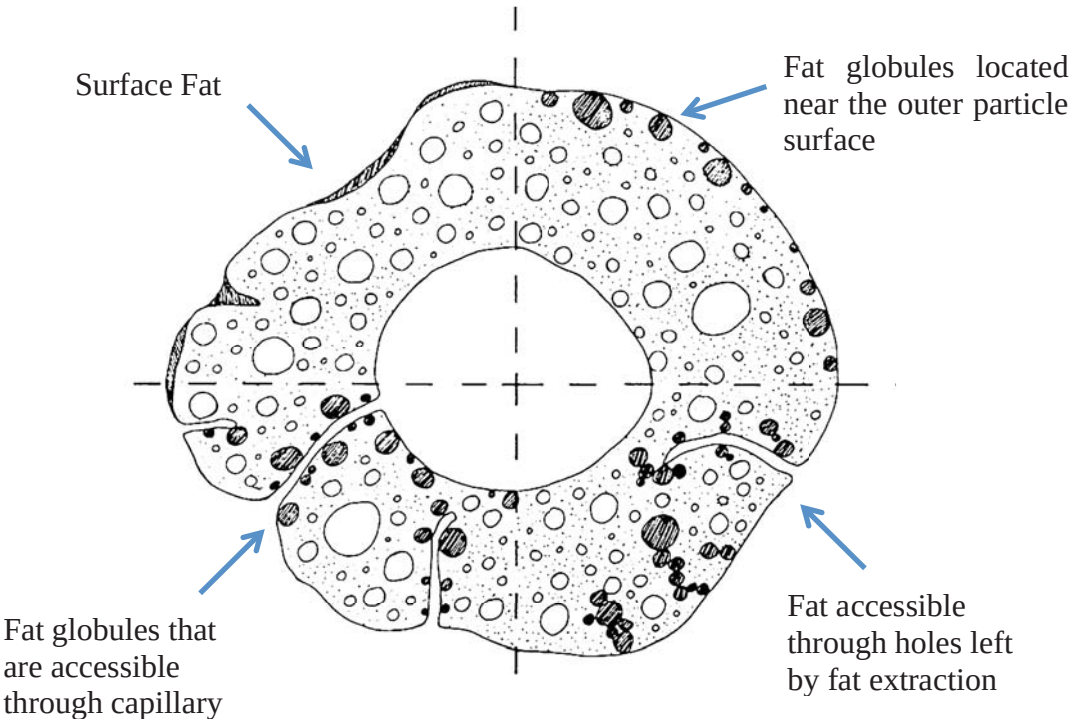


Figure 2.12 *A model depicting the different locations where "free" fat may exist in the powder particle available for extraction using organic solvent (Buma, 1971).*

The first measurements of specific free fat on the surface were undertaken by Faldt et al. (1993) adapting a technique known as electron spectroscopy for chemical analysis (ESCA). By using this technique, identification of elements in the near surface region was possible up to approximately 10 nm depth (Faldt et al., 1993; Vega & Roos, 2006). This technique has since been used by other researchers to study the dried particle surface (Vega & Roos, 2006).

Measurements of surface fat using ESCA have been reported for whole milk powder, which is essentially composed of 30% fat, 30% protein and 40% lactose. But ESCA measurements range varied widely. A 99% coverage of fat on the surface of whole milk powder was reported by Kim et al. (2002) whereas Faldt and Sjoholm (1996) reported a 55% coverage of fat on the surface (Vega & Roos, 2006). As spray drying is a complex operation, difference in powder characteristic may originate as a result of differences in drying conditions and the set up of the dryer e.g. internal fluid bed vs. external fluid bed or atomiser type. However, no correlation has been reported between results collected using ESCA and free fat measurements made using solvent extraction method (Millqvist-Fureby et al., 2001; Vega & Roos, 2006). This was thought to be due to the shortcoming in the two methods. By using ESCA, only 10 nm of the surface can be accurately analysed. This will underestimate the free fat when the fat layer is higher in depth than 10 nm. On the other hand, most methods described in the relevant literature for extracting free fat using organic solvent have a long contact time (~2 min) between the powder particle and the solvent which may overestimate the free fat as free fat from the inside the powder particle through cracks and holes may also be included. A method for extracting surface free fat has been described by Kim et al. (2002) which comprised of a four step extraction process keeping the contact time as short as possible (Vega & Roos, 2006).

2.8.2 Reconstitution

The ease of reconstitution is of the most important properties of manufactured dried emulsions as it directly impacts consumer use. It is important that the emulsions do not change significantly upon spray drying. Freudig et al. (1999) described the reconstitution process as divided into four steps: wetting, submersion, dispersion and dissolving. Wetting is considered to be the rate-controlling step that dictates reconstitution time. Wettability has been defined as the ability of powder to imbibe water when introduced to a liquid due to capillary forces. The factors of powders that may influence the wettability are particle size, density, porosity, surface charge, surface area, the presence of amphiphatic substances and the surface activity of the particles. Powder with a large proportion of lactose on the surface will yield good wettability whereas the presence of fat on the surface may lead to poor wettability (Faldt & Bergenstahl 1996 b; Kim et al., 2002; Vega & Roos, 2006). Large particles wet faster and hence the reason some powders are agglomerated commercially, via lecithinisation in the fluid bed or fines addition to increase the particle size and thus increase wettability (Schubert, 1993; Vega & Roos, 2006). Cream powder wets extremely slowly due to hydrophobic surface properties. Wetting time frame is > 15 min for 1g cream powder in 100 g water. When the same powder was washed with petroleum ether, wetting time decreased to 35 sec (Kim et al., 2002; Vega & Roos, 2006).

2.8.3 Storage stability

The transit time of powder inside the dryer is very small which is why most dry solids produced by spray drying exist in an amorphous state. Dried product would also have hygroscopic and thermoplastic properties which either tend to make the

product stick to the cone of dryer while processing or renders them unstable during storage should moisture or temperature fluctuate. The susceptibility to deterioration for powders containing higher quantities of carbohydrate has been related to their glass transition temperature (Tg) (Aguilera et al., 1995; Christensen et al., 2002, Vega et al., 2005a; Vega & Roos, 2006). Amorphous liquids appear to be solids due to inherent high viscosities. With the increase in temperature, even though the viscosity decreases, they start exhibiting sticky behaviour. With increasing water content, the stickiness is reached at a lower temperature. Therefore, in powders containing high amounts of sugar any combination of temperature, water content and humidity can promote deteriorative processes such as stickiness or sugar crystallisation. Cohesion, defined as inter-particle stickiness, is also linked to the composition of powder and determines the flowability of powders. The decreased flowability (2-fold) of whole milk powder as compared to skim milk powder is contributed by the presence of fat. The presence of water at >6% w/w with or without the presence of lactose can result in the formation of liquid bridges increasing surface stickiness (Rennie et al., 1999; Vega & Roos, 2006).

Caking is another defect affecting powder stability during storage. Free flowing powder can turn into lumpy agglomerated solid or even into a sticky mass, as a result of exposure to high humidity and high temperatures. Lactose crystallisation can also cause caking along with electrostatic attraction between particles. Free fat on the surface of the particle can also cause lumpiness or even caking (Teonou & Fitzpatrick, 1999; Vega & Roos, 2006). Systems where fat is not well encapsulated within the powder matrix will be susceptible to caking during storage.

2.9 Influence of emulsion components and processing condition on stability of powdered emulsions

2.9.1 Influence of emulsion composition

2.9.1.1 Proteins

Several studies have been published on the behaviour of whey protein concentrate or whey protein isolate either alone or in combination with lactose and/or sodium caseinate (Faldt & Bergenstahl, 1996; Keogh & O'Kennedy, 1999, Landstrom et al., 2000; Millqvist-Fureby et al., 2001; Vega & Roos, 2006). The main disadvantage of using whey proteins for emulsification is their susceptibility to heat denaturation and aggregation (Damodaran & Anand, 1997; Vega & Roos, 2006). Faldt and Bergenstahl (1996) studied the encapsulation ability of whey proteins by measuring the amount of oil present at surface of the powder particle after spray drying. They found that 45-60% of the surface of the powder made with whey proteins was covered with oil which was relatively higher compared to investigation made using NaCas (Landstrom et al., 2000; Vega & Roos, 2006). The level of fat in the emulsion also impacted the total free fat and surface fat. Higher levels of fat in the emulsion resulted in higher levels of surface fat (Keogh & O'Kennedy, 1999; Vega & Roos, 2006).

The use of whey protein in combination with NaCas has been shown to increase the stability of emulsions during drying. No significant change in the droplet diameter was noticed before and after drying (Sliwinski et al., 2003). Only in emulsion with more than 70% of whey protein, a shift in the particle size towards larger ranges post spray drying was observed. Sliwinski et al. (2003) also observed the displacement of caseins with whey proteins after spray drying and reconstitution in emulsions, which

had majority of the surface, covered with NaCas before spray drying. The authors attributed this shift in the composition of the oil droplet surface to heat denaturation and aggregation as the amount of β-lactoglobulin increased the most as compared to α-lactalbumin (Sliwinski et al., 2003).

The impact of spray drying on whey protein denaturation and aggregation has not received much attention. However, heating of whey proteins before emulsion formation has been studied by Millqvist-Fureby et al. (2001) under a wide range of conditions. The authors showed that heat treatment of whey proteins before emulsification and drying caused a shift in the droplet diameter towards the larger size range but they did not compare the unheated samples before and after drying. Other studies using whey proteins have shown large surface oil coverage, suggesting that denaturation and aggregation might be less important in whey protein stabilized emulsion powders (Faldt & Bergenstahl, 1996; Vega & Roos, 2006).

Due to the lack of heat sensitivity of caseinates, most studies have shown a lower fat surface coverage in powdered emulsions made using NaCas as compared with whey protein products (Rosenberg & Young, 1993; Young et al., 1993 a, b; Vega & Roos, 2006). When used as an encapsulant on its own, NaCas stabilized emulsions showed a decrease in the microencapsulation efficiency and protein load with an increase in the oil-to-protein ratio. Microencapsulation efficiency has been defined as the ratio of extractable oil measured using a solvent extraction method to the total oil content as measured using the Rose-Gottlieb method (Hogan et al., 2001). The association between surface protein concentration of the emulsion and the surface oil coverage or microencapsulation efficiency has been made rarely in literature (Vega & Roos, 2006). Hogan et al. (2001) also suggested that oil-to-protein ratio was not the only

factor driving microencapsulation efficiency but also the concentration of the filler material played a role. However, the role of matrix forming material in combination with proteins has not been addressed widely. Also, the comparison of different protein materials (monomeric vs. aggregated) has not been made (Vega & Roos, 2006).

2.9.1.2 Carbohydrates

Lactose is exclusively found in milk and is widely used as a matrix forming material in spray dried products. In addition, it widely used in the pharmaceutical industry as an excipient in tablet manufacture. Lactose used in some products goes through a pre-crystallisation stage to avoid caking problems during drying and further storage (Schuck & Dolivet, 2002). However, this is not common for dairy products. Rapid evaporation of water from dairy products means that lactose remains in an amorphous form in finished powders (Vega & Roos, 2006). This lactose in a glassy state is thought to be the main encapsulant of milk fat in dairy-like emulsions (Buma, 1971; Vega & Roos, 2006). Being amorphous, lactose is only stable below its glass transition temperature (Miao & Roos, 2004). Exceeding this, crystallisation of lactose and related processes may impact food quality (Roos & Karel, 1991; Vega et al., 2005). The presence of lactose in spray-dried formulation also has implications during drying, such as stickiness and fouling of the dryer cones (Schuck et al., 2005; Vega & Roos, 2006). Therefore, attention must be paid to choosing the right dryer conditions when using lactose as a bulk ingredient in powdered emulsion formulations (Vega & Roos, 2006). Also, it is critical to have the correct post drying conditions to maintain stability of powders containing lactose as prevention of caking and lumping will depend on the relationship between humidity and temperature (Jouppila & Roos, 1994; Vega & Roos, 2006).

Maltodextrin is a hydrolysis product of starch with a dextrose equivalent of less than 20 (Chronakis, 1998). With a broad range of molecular weight distribution of oligosaccharides, maltodextrins are highly soluble in water with low viscosity. This is the reason for their widespread commercial use as co-encapsulants (McNamee et al 1998; Hogan et al., 2001; Vega & Roos, 2006). Dollo et al. (2003) reported excellent stability during spray drying when a mixture of maltodextrin and NaCas was used to stabilize fractionated coconut oil. No noticeable change was seen in emulsion particle size before and after drying and reconstitution (Vega & Roos, 2006). With increased dextrose equivalent (DE) of maltodextrin in formulation, a significant increase in microencapsulation efficiency was observed. This was attributed to the presence of lower molecular weight carbohydrates, which created a less porous matrix which was less impervious to extraction solvents (Vega & Roos, 2006).

2.9.2 Influence of processing parameters

The characterisation of a dairy powder not only includes the compositional aspect (proteins, carbohydrate, fat, mineral, vitamin and water content) but also include microbiological and physical properties. Some of the important physical properties include bulk density, instant properties, flowability, wettability, whey protein nitrogen index, insolubility index, sinkability index, free fat and interstitial air. All these characteristics have well defined measurement as per international standards (Schuck, 2002). Extensive research has been conducted on spray drying over the past few decades. A large number of studies have demonstrated that the composition of feed (Vega & Roos, 2006; Drusch & Mannino, 2009; Gharsallaoui et al., 2007; Jayasundera et al., 2009) as well as the processing parameters employed (Schuck, 2002; Kelly et al., 2002; Twomey et al., 2000; Verdurmen & Jong, 2003) have a

significant influence on the final powder properties as summarized in Fig. 2.13 (Kim et al., 2009; Verdurmen & Jong, 2003).

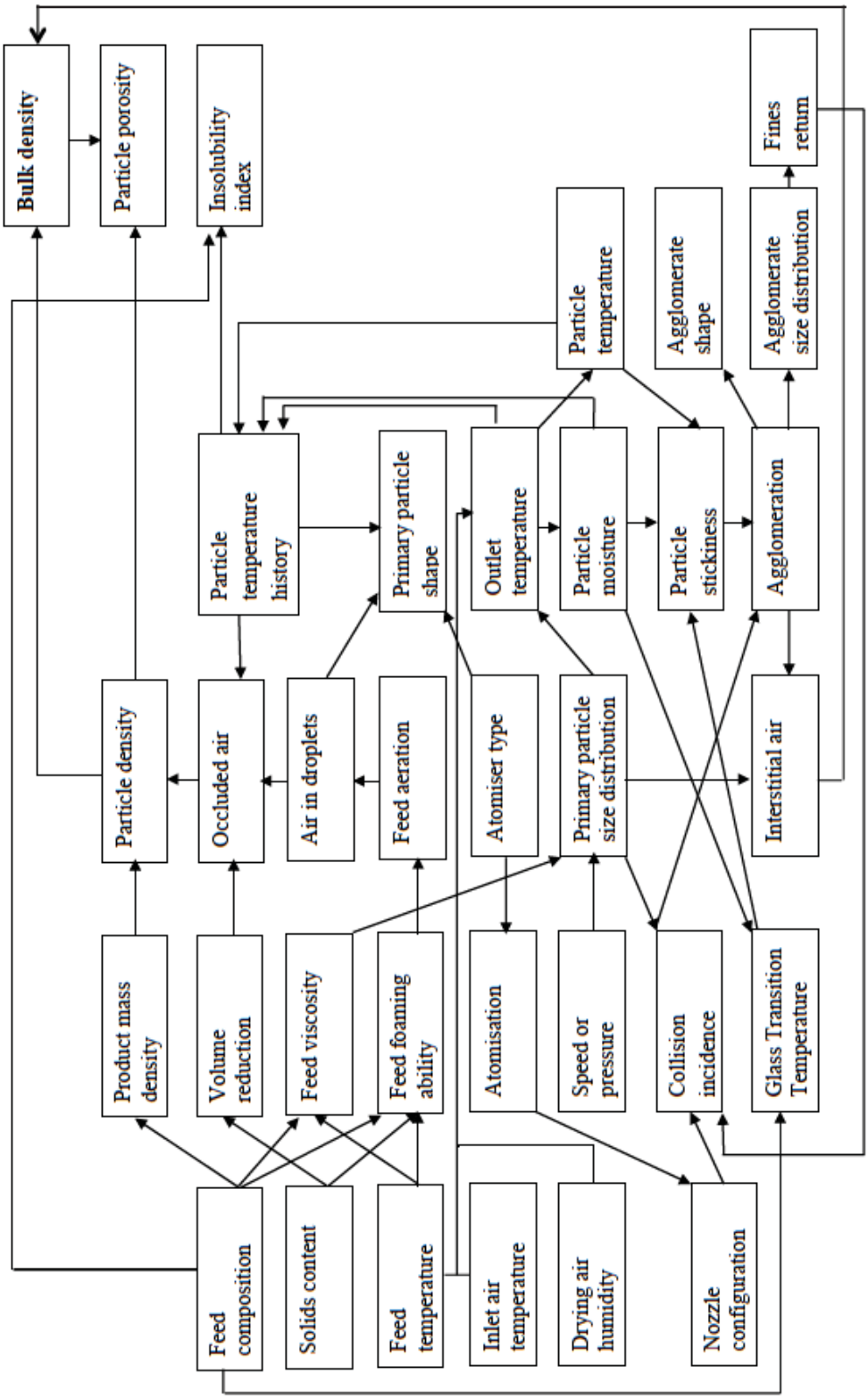


Figure 2.13 Relationship between powder composition and processing parameters on the final powder properties (Verdurmen & Jong, 2003).

2.10 Application of spray drying for microencapsulation of long chain polyunsaturated fatty acids

Spray drying an emulsion does not necessarily result in a microencapsulated ingredient, which can be used in food supplementation or fortification. This is especially true in the case of microencapsulation of long chain polyunsaturated fatty acids (n-3 PUFA). The main reason for poor encapsulation via simple spray drying of an emulsion is the very low threshold of the compounds produced as a result of lipid oxidation to the human senses. Therefore, modification of the formulation as well as the process are required to achieve good microencapsulation of n-3 PUFA. It was thought that the availability of air during the drying process resulted in accelerated oxidation. However, recent study by Serfert et al. (2010) showed that the lipid oxidation in spray dried powders containing n-3 PUFA was attributed to oxygen available in the emulsion rather than the drying gas (Serfert et al., 2010; Drusch & Mannino, 2009). However, the most important element of microencapsulated powders containing n-3 PUFA is surface free fat. Even a small proportion of free fat on the surface may lead to development of off-flavour development as a result of lipid oxidation.

A frequently described technique for the microencapsulation of n-3 PUFA is the use of fluidized bed coating in conjunction with spray drying. The coating has been suggested to act as an added barrier for the surface free fat leading to limited lipid oxidation. Modified starch along with maltodextrin was used as encapsulant and gum arabic was used as coating in the fluidised bed in a patented approach published by Barrier and Rousseau (1997). The use of carnauba wax and paraffin or bees wax and stearic acid has also been used to coat a milk protein-based microcapsule in the

fluid bed (Gautam et al., 2007). Another key microencapsulation approach, which used caseinate-based microcapsules, coated with modified starch served as a benchmark for the food industry for a number of years (Skelbaek & Anderson, 1994; Drusch & Mannino, 2009). In this case, the modified starch served as a secondary coating and could also be achieved in a one-step process by collecting the spray-dried particles in a starch bed (Fig 2.14). It has to be noted that a secondary coating does not necessarily prevent off-flavour development. The secondary coating materials used e.g. waxes; starches and gums may impart sensory defects themselves and thus limit inclusion levels in foods (Drusch & Mannino, 2009). Although, it is well known that the surface oil (free oil) can be minimized by optimisation of emulsion composition as well as the processing parameters (atomiser type, dryer set-up, drying conditions, viscosity of the in-feed), a complete understanding of the mechanisms of how surfaces are formed is still to be obtained (Borrow et al., 2007; Taneja & Singh, 2012).

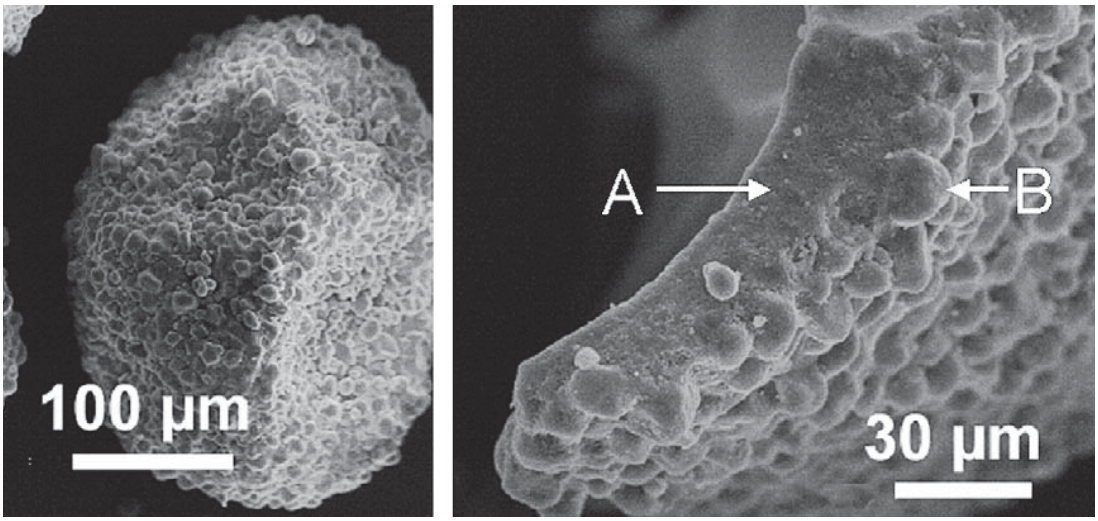


Figure 2.14 *Scanning electron micrographs showing the coating of starch (B) on powder particle surface (A) (Drusch & Mannino, 2009).*

2.11 Objectives

The main objective of this work was to investigate the impact of emulsion components and processing parameter on the stability of these systems during the spray drying process. The experiments in this work were also aimed at understanding the impact of composition and processing parameters on the reconstitution properties as well as storage stability of the resulting powders.

2.11.1 Experimental approach

The experimental studies in this work have been divided into three parts:

1. Compositional impact on emulsion behaviour during drying
    a. Impact of concentration of non-aggregated proteins and maltodextrin on the behaviour of oil droplets during drying.
    b. Impact of concentration of aggregated proteins on the behaviour of oil droplets during drying.
2. Processing impact on emulsion behaviour during drying
    a. Impact of heat treatment of whey proteins before or after emulsion formation on the behaviour of oil droplets during drying.
    b. Impact of combination of whey protein and caseins on the behaviour of oil droplets during drying.
3. Investigation of the correlation reconstitution behaviour and oxidative stability in emulsions made using n-3 long chain polyunsaturated fatty acids.

2.11.2 Experimental techniques

Table 2.2 below provides a brief overview of the different experimental techniques used in the study.

Table 2.2 *Overview of experimental techniques used in the study.*

Study	Technique
Emulsion characterisation	Particle size analysis using laser light scattering
	Sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE)
	Confocal scanning laser microscopy (CSLM)
Powder characterisation	Extractable oil using solvent extraction
	Scanning electron microscopy (SEM)
	Transmission electron microscopy (TEM)
Fat localisation	CSLM

Chapter 3

Materials and equipment

This chapter lists the major materials and equipment used extensively in this study. All methods used in the study have been described at the beginning of the chapters.

3.1 Materials

3.1.1 Whey protein isolate

Whey protein isolate (WPI 895) was provided by Fonterra Cooperative Ltd. Auckland, New Zealand. The composition of the WPI was 94.0 % w/w protein (as is basis); ash 2.8% w/w and moisture 4.23% w/w. The same WPI was used in all experiments.

3.1.2 Sodium caseinate

Sodium caseinate (ALANATE 185) was also provided by Fonterra Cooperative Ltd. Auckland, New Zealand. The composition of the sodium caseinate was 92.0 % w/w protein (as is basis), ash 3.7% w/w; moisture 4.62% w/w and pH of 6.7. The same sodium caseinate was used in all experiments.

3.1.3 Milk protein concentrate

Milk protein concentrate (ALAPRO 4850) was also provided by Fonterra Cooperative Ltd. Auckland, New Zealand. The composition of the milk protein concentrate was 83.1 % w/w protein (as is basis), ash 7.1% w/w and moisture 4.7% w/w. Milk protein concentrate is manufactured from skim milk by using ultrafiltration and diafiltration. The whey proteins present in milk protein concentrate are in native form and caseins are in micellar form similar to that found

in milk (casein 80: whey 20 of the total protein in milk) (Villoslada et al., 2005). The same material was used in all experiments.

3.1.4 Calcium caseinate

Calcium caseinate (ALANATE 385) was also provided by Fonterra Cooperative Ltd. Auckland, New Zealand. The composition of the sodium caseinate was 92.1 % w/w protein (as is basis), ash 3.9% w/w and moisture 3.9% w/w. The same calcium caseinate was used in all experiments.

3.1.5 Soybean Oil

Soybean oil was obtained from Davis Trading Co. (Palmerston North, New Zealand) in 5L bulk can and was stored in the dark at room temperature.

3.1.6 Maltodextrin

Maltodextrin, Fieldose 17GV, used in the study was manufactured by Penford Australia Ltd. and was made by hydrolysis of corn starch. The product had a moisture content 6% (max.) and ash of 0.1% (max.). The dextrose equivalent range of the product was 17-19.9.

Table 3.1 *Compositional details of the various protein products used in this study.*

Component*	WPI	Sodium caseinate	Calcium caseinate	MPC
Protein (%)	94	92	92.1	83.1
Fat (%)	0.29	0.8	1.5	1.6
Lactose (%)	0.4	0.2	0.1	4.3
Moisture (%)	4.23	4.62	3.9	4.7
Ash (%)	2.8	3.7	3.9	7.1

* All data presented in table above is on “as is” basis. “As is” basis for dairy ingredients refers to compositional data including moisture. “Dry basis” on the other hand refers to compositional data excluding moisture.

3.1.7 Chemicals

All chemicals used in the study were of analytical grade with 99% minimum purity. Chemicals used have been listed in Table 3.1.

Table 3.2 *List of chemicals and reagents and their suppliers used in this study.*

S. No	Chemical/Reagent	Product reference number
1	Sodium Chloride (NaCl)	S7653
2	Tris (hydroxymethyl) aminomethane (tris)	252859
3	Sodium dodecyl sulphate (SDS)	L3771
4	Glycine	G8898
5	Acrylamide	8887
6	N,N’-methylene-bis-acrylamide	146072
7	Bromophenol blue	B0126
8	Coomassie brilliant blue R-250	B0770
9	Ammonium persulphate (APS)	A3678
10	N,N,N,’,N’-tetramethylethylenediamine (TEMED)	T9281
11	2-propanol	1.09634.5000*
12	Glacial acetic acid	1.00063.2500*

13	Molecular weight markers for SDS PAGE 10kDa to 250kDa	1610363**
14	Glycerol	G6279
15	Hydrochloric acid (HCl)	1.00317.100
16	Sodium azide	S8032

Sigma-Alderich Ltd., St. Louis, USA

*Merch KgaA, Darmstadt, Germany

**Bio-rad Laboratories, USA

3.2 Equipment

In the following section, details of the equipment used in the study have been detailed.

3.2.1 Centrifuges

Different centrifuges were used for processing samples at different speeds. For samples to be centrifuged at <5000g (used for SDS PAGE), a bench top centrifuge (Thermo Scientific, Lanenselbold, Germany) was used. For speeds of >5000g, a Sorvall RC5C ultracentrifuge was used (DuPont Co., Wilmington, DE, USA) with T-890 rotor. The samples were centrifuged in Nalgene Oak Ridge centrifuge tubes with aluminium caps.

3.2.2 Water bath

Samples were heated using a water bath with temperature-controlled heating coil (Jeio Tech, South Korea). The accuracy of the temperature display was ± 0.1°C. While in use, the temperature of the water and the temperature of the product was checked using a hand-held thermometer (thermocouple type) as well as a glass mercury thermometer.

3.2.3 pH meter

pH meter used in the research was supplied by Oakton Instruments (Illinois, US). The pH meter was calibrated before measurement (daily) by using standard buffer solutions of pH 4 and 7. While not is use the pH meter probe was left in a 3M storage solution of potassium chloride.

3.2.4 Hand held homogeniser

Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operating at 13,500 rev min^{-1} was used in the preparation of primary emulsions.

3.2.5 Microfluidizer

The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). The microfluidizer was fitted with an interaction chamber 75 μm wide with a 'Y' type slot (F12Y) in line with an auxiliary processing module (APM) to provide back pressure in the line (Fig. 3.1).

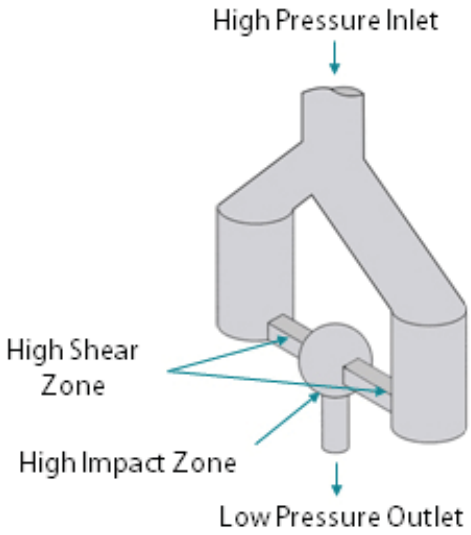


Figure 3.1 *Illustration of a Y-type single slotted interaction chamber used in the preparation of the emulsions in this study.*

3.2.6 Spray drier

The spray drier used in the research was a Buchi B-290 (Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current configuration and fitted with a two-fluid nozzle (Fig. 3.2) (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The unit was at all times attached to a dehumidification unit (B296) to have better control over moisture content and throughput. Both the compressed air unit and the air inlet of the dehumidifier unit were fitted with air filters.

Figure 3.2 *Image of a B-290 bench top spray drier manufactured by Buchi Laboratories, Flawil, Switzerland.*

3.2.7 Particle size

The diameters of the oil droplets for both the parent emulsions and the redispersed emulsions were determined by the laser light scattering using a Mastersizer 2000 E (Malvern Instruments, Malvern, Worcestershire, UK).

Figure 3.3 *Image of the Mastersizer 2000 E manufactured by Malvern Instruments, Worcestershire, UK.*

3.2.8 Confocal scanning laser microscope

Confocal scanning laser microscopy (SP5 DM6000B, Leica Microsystems, Heidelberg, Germany) of emulsions was carried out using a 63 X oil immersion objective with an excitation line of 488 nm from an argon laser. For powders, a 100 X oil immersion objective with sequential excitation lines of 488nm from argon laser and 633nm from a helium-neon laser was used. Details regarding sample preparation have been provided in the chapters where confocal microscopy was used.

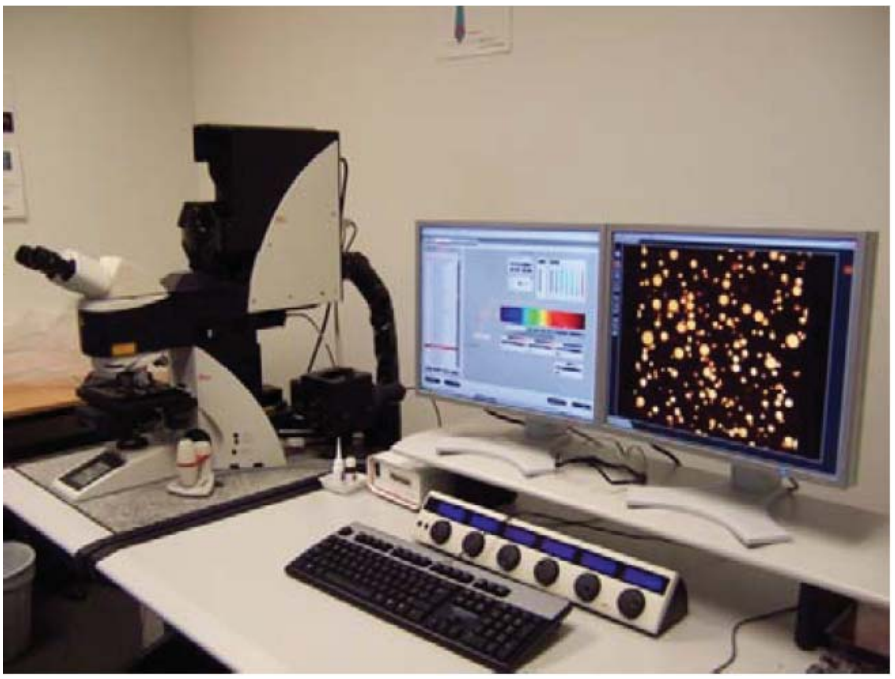


Figure 3.4 *Image of a confocal scanning laser microscope model SP5 DM6000B manufactured by Leica Microsystems, Heidelberg, Germany.*

3.2.9 Scanning electron microscope

Imaging of morphological and structural properties of the powdered emulsions was performed using an FEI Quanta 200 scanning electron microscope (FEI Electron Optics, Eindhoven, Netherlands) was used.

3.2.10 Transmission electron microscope

The imaging of the section grids was undertaken on a Phillips CM10 transmission electron microscope (Eindhoven, The Netherlands) operating at 80 kV.

Chapter 4

Behaviour of oil droplets during spray drying of milk-protein-stabilized oil-in-water emulsions

4.1 Abstract

The effects of spray drying on the behaviour of oil droplets in oil-in-water emulsions (12.0%, w/w, maltodextrin, 20.0%, w/w, soybean oil) stabilized with either sodium caseinate or whey protein isolate (WPI) were examined as a function of protein concentration (0.5−5.0%, w/w). Spray drying and redispersion caused a shift in the droplet size distribution to larger values for all emulsions made using low protein concentrations (0.5−2.0%, w/w), in comparison with their respective parent emulsions. However, the droplet size distribution was affected only very slightly by spray drying when the protein concentration was above 2.0% (w/w). The effects of maltodextrin concentration (1.0−25.0%, w/w) on the behaviour of WPI-stabilized emulsions (0.5−10.0%, w/w, WPI, 20.0%, w/w, soybean oil) were also examined. Emulsions containing low levels of maltodextrin showed marked re-coalescence during spray drying and redispersion even at a WPI concentration of 10.0% (w/w).

The contents of this chapter have been published in a peer reviewed journal article and have been adapted with permission. Please refer to page 265.

4.2 Introduction

Milk proteins are widely used in manufactured foods because of their excellent surface-active and colloid-stabilizing characteristics (McClements, 2010; Singh & Ye, 2009; Vega & Roos, 2006). These food products may be in emulsion form, either in the final product (e.g., ice cream, nutritional beverages, creams, sauces etc.) or at some stage of manufacture (infant formulae, ice cream premixes, cream powders, coffee creamers etc.) (McClements, 2010). Because of their amphiphilic nature, milk proteins are readily adsorbed at the freshly formed interface of the oil droplet during emulsification and create repulsive interactions between droplets, facilitating the stabilization of oil droplets in emulsions (Singh, 2011; Ye, 2008). Extensive research has been carried out to understand the adsorption process, the composition and the structure of the adsorbed protein layers and how they influence the physical and chemical properties of emulsions (Singh, 2011). However, there is as yet no comprehensive understanding of the changes taking place in such systems during drying and upon redispersion (Vega & Roos, 2006).

Drying into a powdered format is a convenient means of increasing the shelf life of perishable emulsions and is generally carried out using spray drying (Vega & Roos, 2006). It is important that, on redispersion after spray drying, the droplet size distribution of the emulsion remains the same as that before drying (parent emulsion). This would indicate that the emulsion droplets were not disrupted during drying and were well preserved in the powder matrix (Faldt & Bergenstahl, 1996; Jafari, Assaidpoor, Bhandari, & He, 2008; Vega & Roos, 2006). However, re-coalescence of emulsion droplets during drying has been reported (Hogan,

McNamee, O'Riordan, & O'Sullivan, 2001a, b; Jafari et al., 2008), causing an increase in the droplet size upon redispersion.

The physical and chemical properties of powdered emulsions, e.g., dispersibility, wettability and oxidative stability, are determined by the physical structure and the surface composition of the powder particles, which are partly affected by the emulsion stability during drying (Drusch & Schwarz, 2006; Faldt & Bergenstahl, 1996; Hogan et al., 2001a; Landstrom, Alsins, & Bergenstahl, 2000). Powders with high oil content, e.g., whole milk powder and cream powder, have been reported to have the majority of their surfaces covered with fat, resulting in a surface composition that is different from the bulk composition, suggesting segregation between the components during spray drying (Kim, Chen, & Pearce, 2003; Nijdam & Langrish, 2006). This surface oil would be readily available for oxidation, reducing the quality of the finished product, and would also result in poor redispersion of the powder in water (Drusch & Berg, 2008; Kim, Chen, & Pearce, 2008). The composition of the powder surface is thought to be largely determined by the constituents in the parent emulsion and the processing conditions (Millqvist-Fureby, Elofsson, & Bergenstahl, 2001).

Most of the published research on milk-protein-stabilized emulsions has been focused on pure emulsions consisting of water, oil and emulsifier, and the stability of the emulsion and the behaviour at the oil/water interface has been evaluated. Subsequent spray drying requires the addition of a hydrophilic carrier material, which has both a direct influence and an indirect influence on the stability of the emulsion (Chang & Pikal, 2009; Faldt & Bergenstahl, 1996; Jafari, He, & Bhandari, 2007). Moreover, there have been only a limited number of studies on the spray drying of emulsions with high oil content (> 20% dry basis). In the present study,

we compared the behaviour of oil droplets during spray drying and redispersion in oil-in-water emulsions stabilized by whey protein isolate (WPI) or sodium caseinate (NaCas) at a range of protein concentrations was studied. The effects of spray drying on the properties of the emulsions stabilized by whey protein as a function of protein and maltodextrin (MD) concentration were also explored.

4.3 Materials and methods

Whey protein isolate (WPI), sodium caseinate (NaCas), soybean oil and maltodextrin (MD) were used in this study. The details regarding these materials and the equipment used in the study have been described in Chapter 3 (section 3.1 and 3.2). A process flow diagram of emulsion preparation, spray drying and analyses for this study has been presented in Figure 4.1.

4.3.1 Emulsion preparation

Proteins (WPI or NaCas) were dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solution was stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solution was then adjusted to 7.0 using 1M HCl or 1M NaOH if required. Similarly, MD solution was prepared using the same stirring equipment with stirring until the powder was dissolved completely. Soybean oil at 20.0% (w/w) was blended with the aqueous solutions of protein and MD using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Approximately, 500 mL of emulsion was prepared for each variation for spray drying purposes.

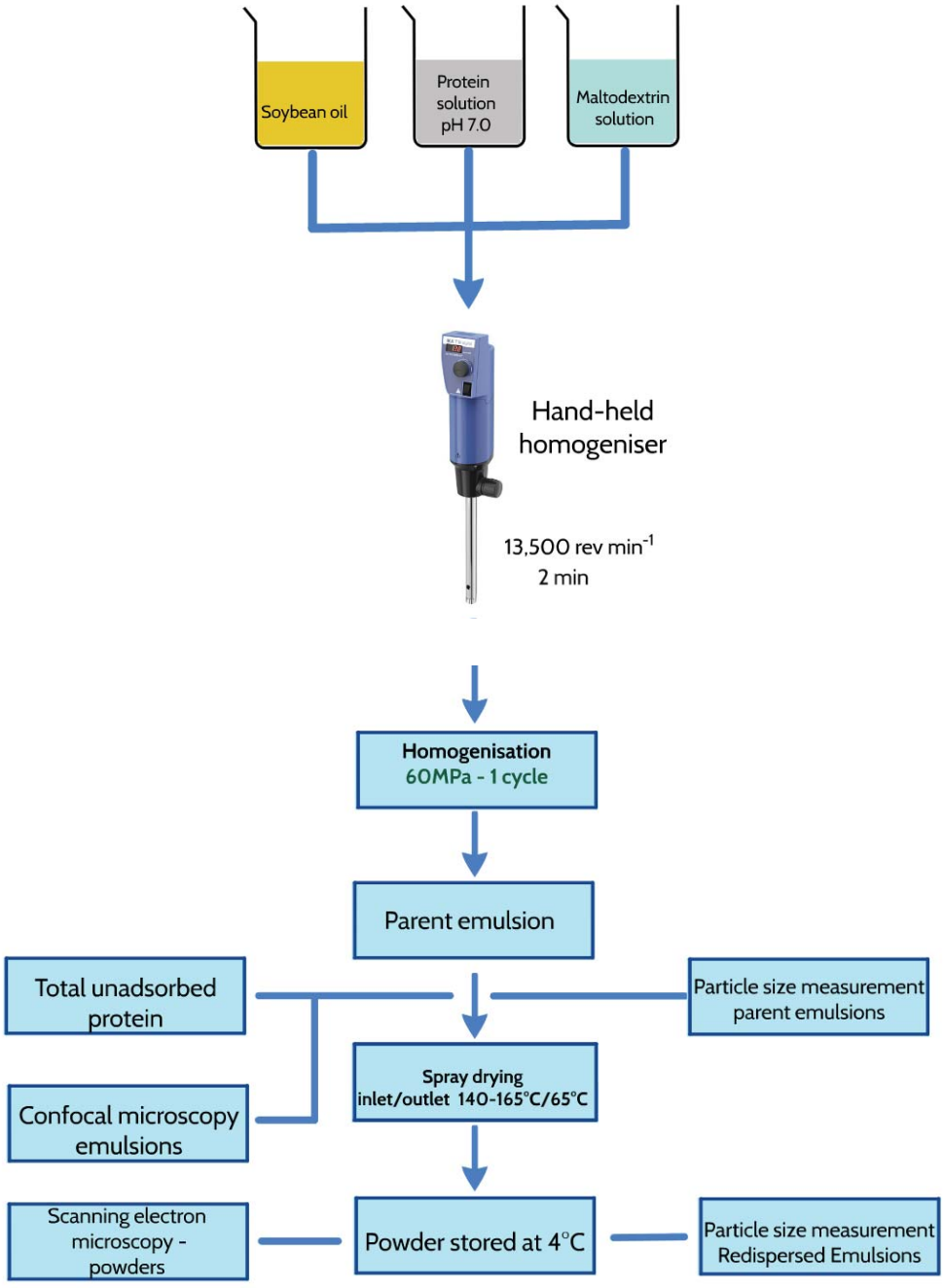


Figure 4.1 *Process flow diagram showing the emulsion preparation, spray drying and analyses for this study.*

4.3.2 Spray drying

All emulsions were spray dried using a Buchi bench-top spray drier (B-290, Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current

configuration and fitted with a two-fluid nozzle (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The inlet temperature varied between 140 and 165 °C and the outlet temperature was maintained at 65 °C. The resulting powders were collected in air-tight containers, allowed to cool to ambient temperature and then stored at 4 °C for further analysis.

4.3.3 Determination of average droplet diameter

Size distribution of the parent emulsions (fresh emulsions which have not been spray dried) and the redispersed emulsions (emulsions prepared by dissolving spray dried powdered emulsions in RO water) were determined by the laser light scattering method as described by Jafari et al. (2007) and Ye (2011) using a Mastersizer 2000 E (Malvern Instruments, Malvern, Worcestershire, UK). Redispersed emulsions were prepared by reconstituting the dried emulsion powder in RO water to obtain the same total solids content as the parent emulsion. Powder was slowly added to RO water and the dispersions were stirred for at least 30 min before particle size measurements were made. In some cases, the emulsions were diluted 1:1 with 2.0% (w/w) sodium dodecyl sulphate (SDS) solution before the measurements were made. The comparison of the size distributions in RO water alone and with the addition of SDS indicated flocculation/aggregation during emulsification/redispersion (Sanchez & Patino, 2005).

The average droplet diameter is commonly expressed as either the Sauter (surface area weighted) or volume weighted average droplet diameter (also written as d_{32} or d_{43} values, respectively):

$$d_{32} = \sum n_i d_i^3 / \sum n_i d_i^2 \quad (1)$$

Sauter (surface area weighted) average droplet diameter

$$d_{43} = \sum n_i d_i^4 / \sum n_i d_i^{32} \quad (2)$$

Volume weighted average droplet diameter

where, n_i is the number of droplets with diameter d_i in the emulsion.

In a monodispersed colloidal system, d_{32} and d_{43} values will be same. However, in a polydispersed system, volume weighted average diameter or d_{43} values were suggested to be more sensitive to the coalescence or flocculation as compared to surface weighted average diameter or d_{32} values (McClements, 2015). Therefore, in this study, volume weighted average diameter or d_{43} values have been used for presented the average droplet diameter of emulsions.

4.3.4 Total unadsorbed protein concentration

The total unadsorbed protein concentration was determined using the method of Ye (2008) with some modifications. Emulsions (30 g) were centrifuged at 45,000 *g* for 45 min at 20 °C in a temperature-controlled ultracentrifuge (Sorvall RC5C, DuPont Co., Wilmington, DE, USA) and the subnatant were carefully removed using a syringe. The cream layer was dispersed in RO water and re-centrifuged at 45,000 *g* for 45 min. Again, the subnatant and the cream layer were collected carefully. Each subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corporation, Billerica, MA, USA). The filtrates were analysed separately for total protein using the Kjeldahl method (1026 Distilling Unit and 1007 Digestor Block, Foss Tecator AB, Höganäs, Sweden). A factor of 6.38 was used to convert nitrogen to protein content. The total unadsorbed protein concentration (%) was calculated

by adding the percentage protein concentrations in each of the subnatant collected after centrifugation.

4.3.5 Confocal scanning laser microscopy

About 500 μL of emulsion (both parent and redispersed) sample was taken in an Eppendorf tube, 10 μL of Nile red (fluorescent dye, 0.1% w/v in acetone) was added and the sample was gently shaken. A volume of 60 μL from this sample was placed on a concave microscope slide. A cover slip was carefully placed and the sample was observed under the microscope. A 63 X oil immersion objective with sequential excitation lines of 488 nm from an argon laser was used.

For powder samples, the fluorescent dyes were dissolved in the oil phase (Nile red, 0.02%, w/w) and the protein solution (Fast green, 0.01%, w/w) followed by emulsion preparation and spray drying (Fig. 4.2). The resulting powder was carefully mounted on to a concave microscope slide using DPX (non-aqueous mounting medium). A cover slip was placed carefully on top, avoiding air bubbles. A 100 X oil immersion objective with sequential excitation lines of 488 nm from an argon laser and of 633 nm from a helium−neon laser was used. All images were acquired at 20 °C (± 2 °C).

4.3.6 Data analysis

Each data point represents average value of two determinations on two separate emulsions (measured in duplicates). Error bars in graphs represent standard deviation of average values of two determinations on separate emulsions. All graphs were drawn using Sigmaplot 13 graphing and data analysis software (Systat Software Inc., San Jose, CA, USA).

Figure 4.2 *A glass bottle showing Nile red mixed with the oil phase (top layer, fluorescent orange) and fast green mixed in the water phase (bottom phase) before emulsification of the mixture. These emulsions were specifically produced for confocal scanning laser microscopy.*

4.4 Results and discussion

Figs. 4.3a and 4.3b show the droplet size distributions of emulsions (12.0%, w/w, MD, 20.0%, w/w, soybean oil) containing WPI or NaCas with concentrations ranging from 0.5 to 5.0% (w/w). The droplet size distributions of the emulsions made using WPI or NaCas were comparable before drying (parent emulsions) and showed bimodal distributions that became broader with a decrease in the protein concentration from 5.0 to 0.5% (w/w).

The average droplet diameter (d_{43}) values of the parent emulsions showed no marked change with a decrease in the concentration of WPI or NaCas in the aqueous phase from 5.0 to 1.0% (w/w). With a further decrease in the protein concentration to 0.5% (w/w), there was an increase in the average diameter values (Figs. 4.4a and 4.4b). When these parent emulsions were diluted with 2.0% (w/w) SDS solution, there was no noticeable change in the droplet size distribution or the d_{43} values (results not shown), indicating that the increase in droplet size at a protein concentration of 0.5% (w/w) was due mainly to coalescence rather than to aggregation/flocculation.

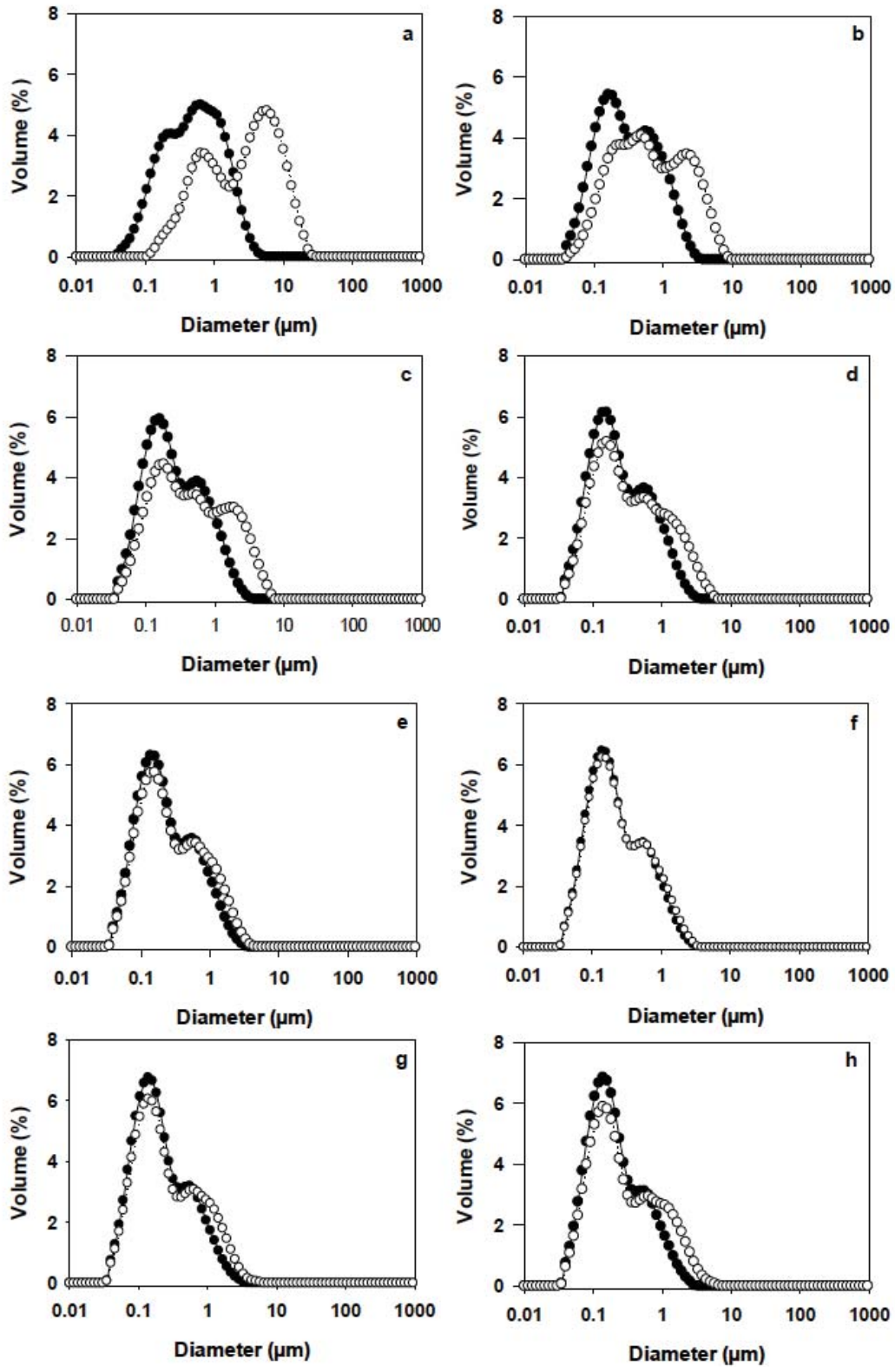


Figure 4.3a *Droplet size distributions of emulsions (0.5−5.0% w/w WPI, 20.0% w/w soybean oil, 12.0% w/w MD) before spray drying (●) and after redispersion (○); whey protein isolate concentrations were; a, 0.5%; b, 1.0%; c, 1.5%; d, 2.0%; e, 2.5%; f, 3.0% g, 4.0%; h, 5.0%.*

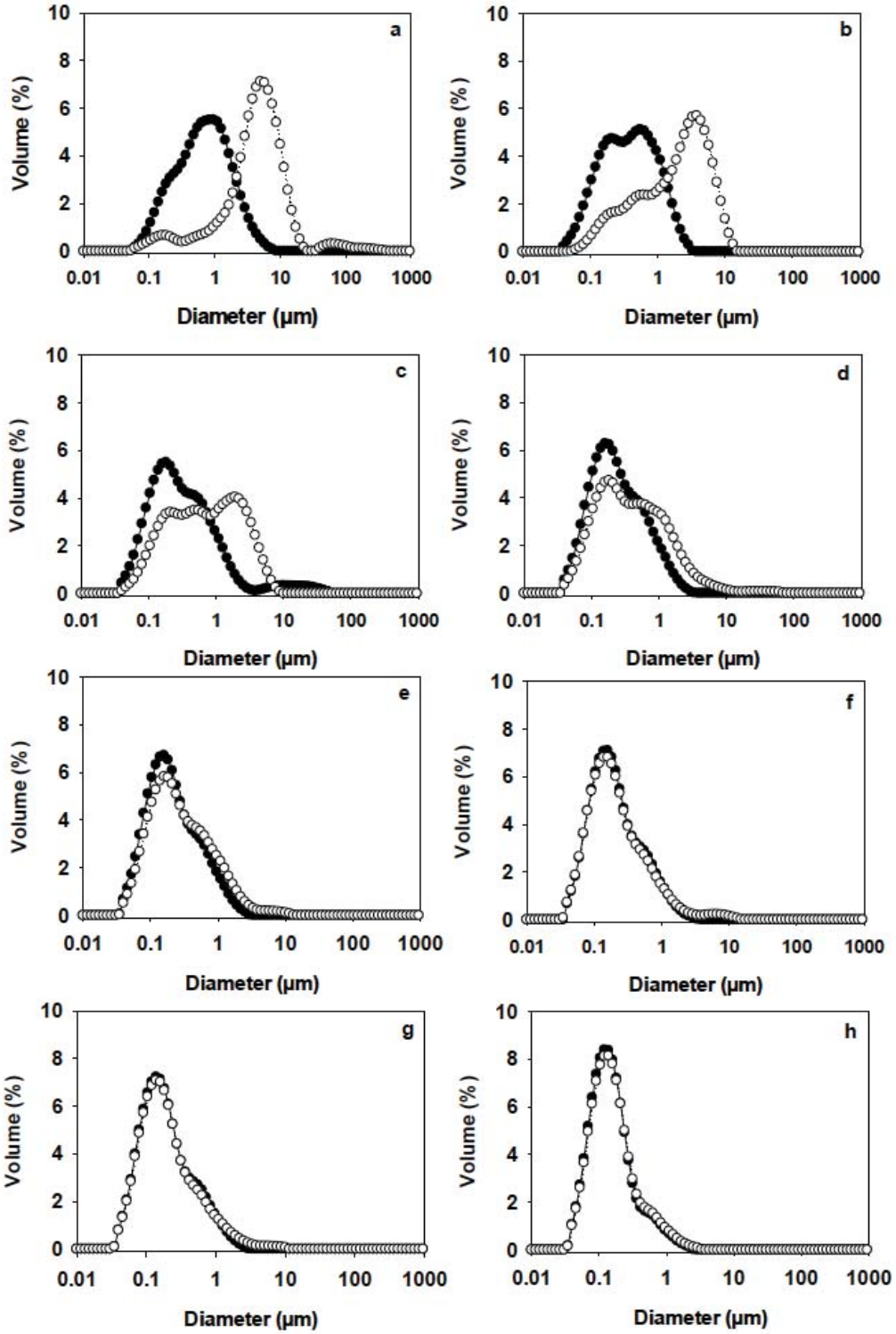


Figure 4.3b *Droplet size distributions of emulsions (0.5−5.0% w/w NaCas, 20.0% w/w soybean oil, 12.0% w/w MD) before spray drying (●) and after redispersion (○); sodium caseinate concentrations were; a, 0.5%; b, 1.0%; c, 1.5%; d, 2.0%; e, 2.5%; f, 3.0% g, 4.0%; h, 5.0%.*

The increase in d_{43} with a decrease in the protein concentration from 1.0 to 0.5% (w/w) was probably related to insufficient protein being available for adsorption at the newly formed droplet interfaces, thus leading to coalescence. Coalescence, which is always irreversible, results from the rupture of the stabilizing film at the interface, leading to an increase in droplet size (Singh & Ye, 2009; Walstra, 2003).

Upon spray drying and redispersion of the powder in RO water, the size distributions for emulsions stabilized by both WPI and NaCas shifted towards a larger size range, compared with the parent emulsions, at protein concentrations between 0.5 and 2.0% (w/w) (Figs. 4.3a and 4.3b). Some aggregation in these emulsions was seen because the d_{43} values shifted towards a smaller size range when the emulsions were mixed 1:1 with 2.0% (w/w) SDS solution (Figs. 4.4a and 4.4b). However, the majority of the shift in the droplet diameter post spray drying, compared with the parent emulsion, was due to re-coalescence. Redispersed emulsions containing protein concentrations between 2.5 and 5.0% (w/w) showed only a very slight change in the size distributions, compared with the parent emulsions, indicating good droplet stability during the drying process. The d_{43} values were consistent with the size distribution curves, showing similar and small values between 2.5 and 5.0% (w/w), compared with the parent emulsions. Under these emulsification and drying conditions, a WPI concentration of 3.0% (w/w) appeared to be optimum for stabilizing the emulsion droplets during spray drying because the droplet distributions nearly overlapped. For NaCas-containing emulsions, protein concentrations > 3.0% (w/w) showed overlapping distributions before and after spray drying.

For coalescence to take place, emulsion droplets must be close to each other. When proteins are used as stabilizers in oil-in-water emulsions, they adsorb at the

oil/water interface, providing electrostatic repulsion, which acts between emulsion droplets. This prevents the close approach of emulsion droplets, which in turn reduces the chances of coalescence (Walstra, 2003). Film rupture, the main reason for droplet coalescence in emulsions, is known to occur rarely in oil-in-water emulsions stabilized by proteins with a small droplet size (< 1 μm) and having a saturated interface because the interfacial film has very low mobility. In contrast, emulsions stabilized using low molecular weight surfactants rely on the Gibbs−Marangoni mechanism to stabilize interfaces because of their high mobility. However, if emulsions stabilized with surfactants have a very thin film (a few nanometres), a hole can form in the interface spontaneously. Once this happens, the neck of the hole will grow very rapidly, resulting in two droplets and forming one bigger droplet. It should be noted that these mechanisms are based on aqueous systems, in which external stresses, such as colloidal attractions and shear stress, force droplets close to each other. In such systems, stability towards coalescence is dependent on the ratio of the external stress to the internal stress exerted within the emulsion droplet (Laplace pressure) (Walstra, 2003).

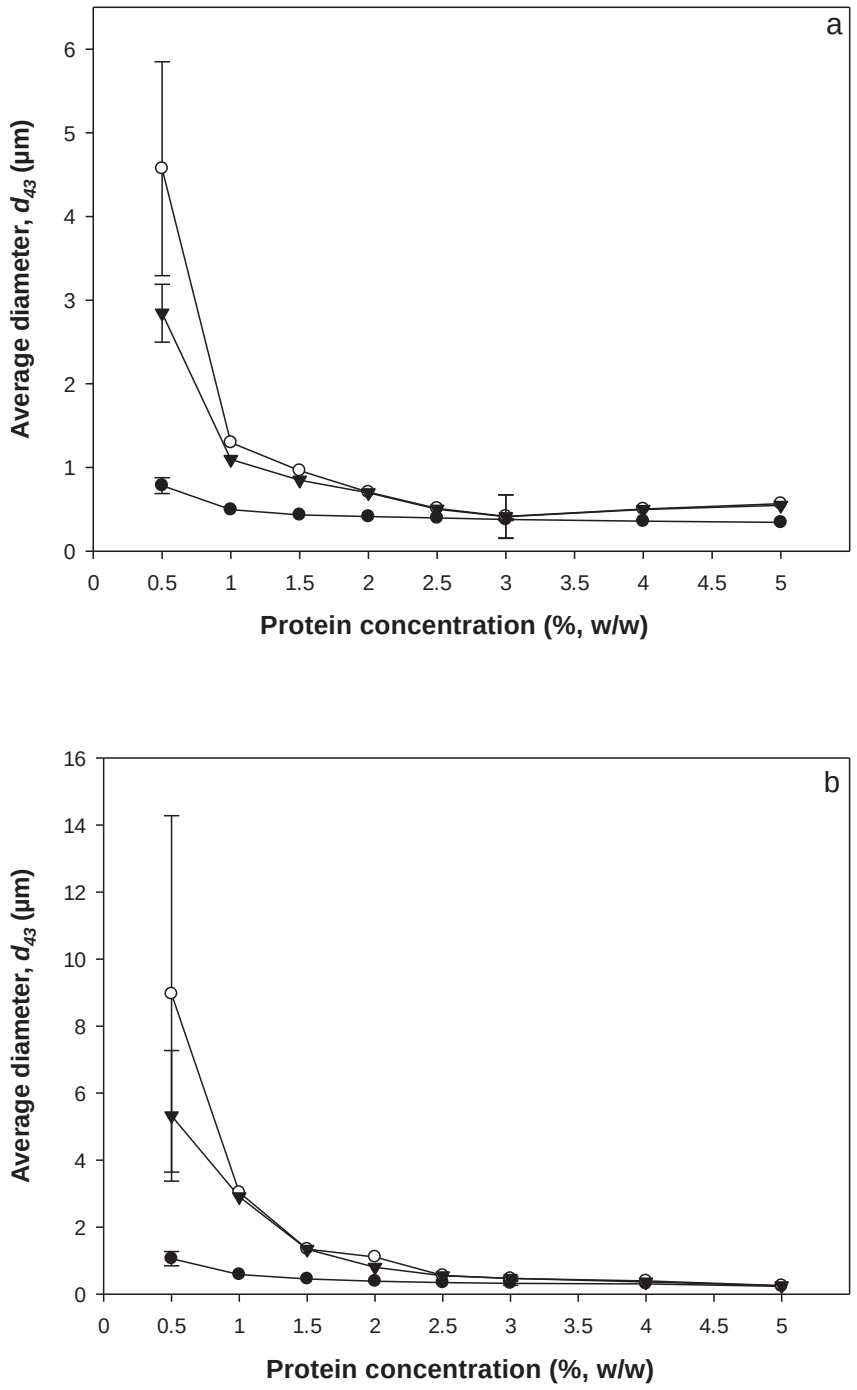


Figure 4.4 *Average droplet diameter (d_{43}) values of parent (●) and redispersed (○) emulsions containing 20.0% w/w soybean oil, 12.0% w/w MD and 0.5−5.0% w/w WPI (a) or NaCas (b). The redispersed emulsions were also diluted (1:1) with 2.0% w/w SDS solution (▼).*

However, under extreme conditions such as spray drying, emulsion droplets are forced close to each other as the water is removed from the drying droplet (Walstra, 2003). Processes such as high shear and the creation of an immense air/liquid interface during atomization can impose high stress on the adsorbed interfacial proteins (Elversson & Millqvist-Fureby, 2005). In this case, the composition of the aqueous phase and the thickness of the interface will probably be the most important variables that affect the stability of the droplets. To confirm that the dehydration processes, rather than the atomization step, were mainly responsible for re-coalescence, the size distribution of the emulsion collected immediately after atomization when no heat dehydration was applied in the spray-drying chamber were measured. The droplet size distributions of parent emulsions containing either WPI or NaCas at protein concentrations of 0.5−2.0% (w/w) did not change after atomization (results not shown), indicating that stresses induced by atomization were not responsible for re-coalescence in these emulsions.

The minimum limiting concentration of adsorbed proteins required to stabilize an emulsion is considered to be slightly lower for NaCas (~ 1 mg m^{-2}) than for WPI (~ 1.5 mg m^{-2}) (Hunt & Dalgleish, 1994). This corresponds to a total protein concentration in the continuous phase of ~ 1.0% (w/w) for WPI and slightly lower for NaCas (Hunt & Dalgleish, 1994). However, it is well known that, above a maximum obtainable surface coverage, both whey proteins and caseins can associate loosely at the interface, forming multiple layers (Euston & Hirst, 1999). The total unadsorbed protein concentration (%) for both parent emulsions, containing WPI or NaCas was measured, as the excess protein in the continuous phase has been suggested to have a possible stabilizing role in emulsions during spray drying (Hogan et al., 2001b; Vega & Roos, 2006).

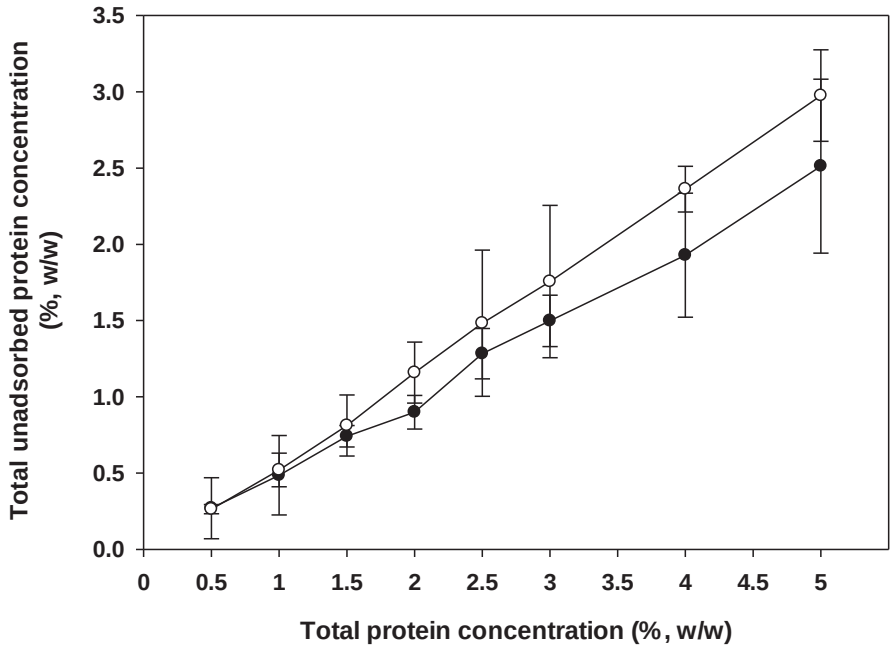


Figure 4.5 *Total unadsorbed protein concentration (%, w/w) in the bulk phase of the parent emulsions containing either WPI (●) or NaCas (○) as a function of the total protein concentration (%, w/w).*

At protein concentrations below 2.0% (w/w), the results for total unadsorbed protein for emulsions containing WPI or NaCas were comparable (Fig. 4.5). At protein concentrations between 2.0 and 5.0% (w/w), NaCas-containing emulsions had a slightly higher unadsorbed protein concentration than WPI-containing emulsions. This was due to the highly flexible structure of the casein molecules, which respond to the change in protein concentration better than WPI, and casein molecules appear to adsorb at the oil interface in a more efficient manner (Euston & Hirst, 1999). The results indicate that ~ 1.0% (w/w) total unadsorbed protein (WPI or NaCas) in the bulk phase was essential to prevent re-coalescence of the emulsion droplets during spray drying, as indicated by the d_{43} values of the redispersed

emulsions. Above this concentration of unadsorbed protein in the bulk phase, the size distributions of the redispersed emulsions were similar to those of the parent emulsions, indicating no droplet coalescence (Figs. 4.3a, b and 4.4).

In recent years, confocal microscopy has been used to study the localization of fat droplets in dairy powders (Auty, Twomey, Guinee, & Mulvihill, 2001; Kim, Chen, & Pearce, 2002; McKenna, 1997; Ye, Anema, & Singh, 2007). The confocal micrographs of the emulsion powders showed good agreement with the size distribution results; powder particles containing 0.5% (w/w) WPI indicated coalesced oil droplets, whereas powder particles containing 3.0% (w/w) WPI indicated that the oil droplets were small and evenly distributed (Figs. 4.6 a and b). This implied that the coalesced droplets were located inside the matrix and that re-coalescence was probably taking place only during the dehydration process. It was interesting to observe that the surfaces of the powder particles in the powders containing both 0.5 and 3.0% (w/w) protein were dominated by proteins (represented by green colour), highlighting the surface-activity-driven diffusive movement of proteins towards the powder surface, which has been widely reported in the literature (Adhikari, Howes, Bhandari, & Langrish, 2009; Faldt & Bergenstahl, 1994).

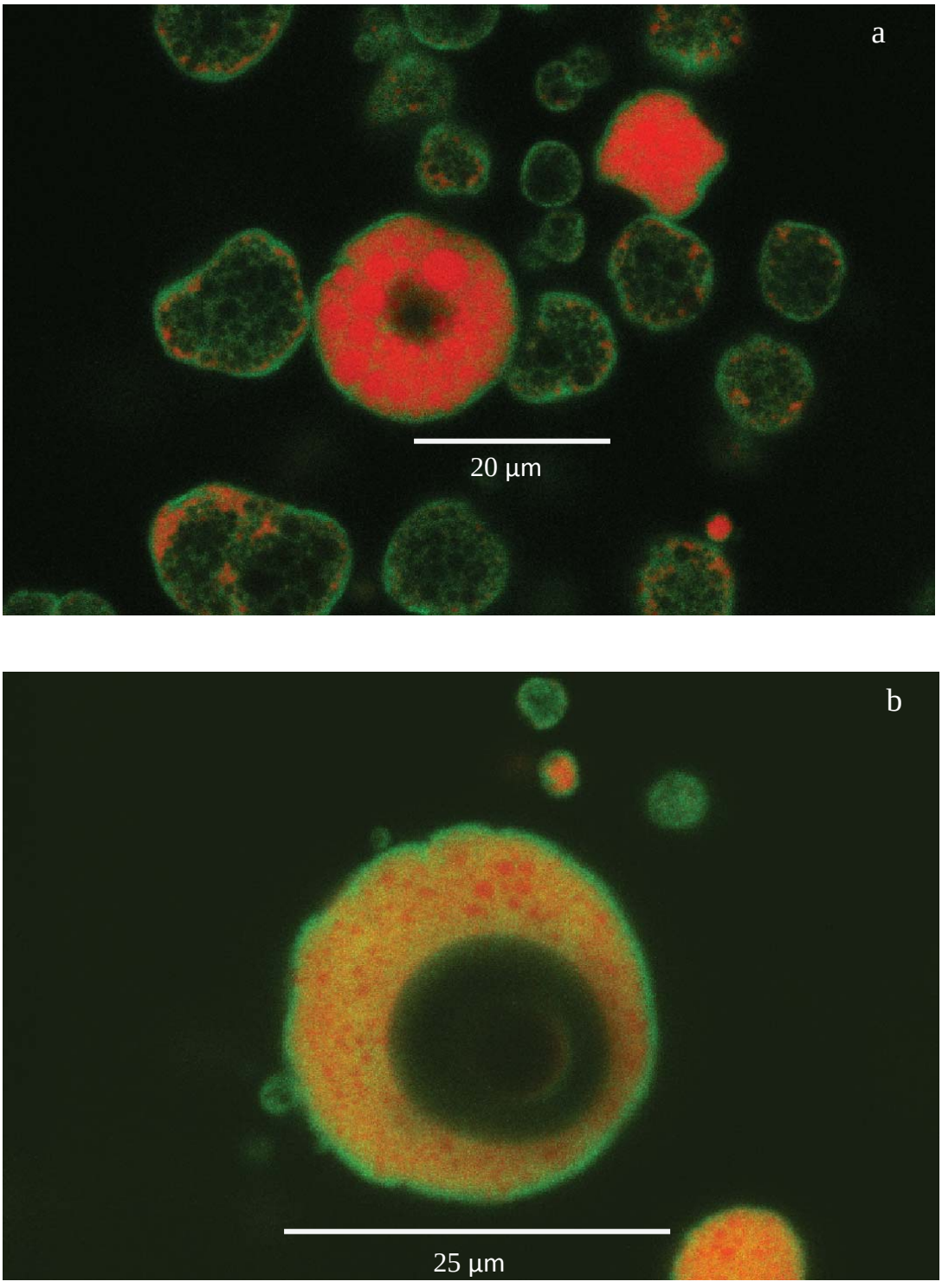


Figure 4.6 *Confocal micrographs of powdered emulsion particles (20.0% w/w soybean oil, 12.0% w/w MD) stained with Nile red and fast green. (a) 0.5% w/w WPI and (b) 3.0% w/w WPI. Red represents the oil phase and green represents whey proteins.*

The creation of the air/water interface during spray drying and the subsequent removal of water cause protein to migrate from the bulk to the air/water interface. At low protein concentration, it is likely that some of the adsorbed protein molecules migrate to the air/water interface, as there is insufficient protein in the bulk phase to fully cover the newly created air/water interface. This could create "gaps" in the interface, which may lead to re-coalescence of the oil droplets. As expected, this effect would be less at higher concentrations of protein in the continuous phase. It should be noted that the emulsions in the present study contained 12.0% (w/w) MD, which has been shown to lead to the formation of a solid matrix during spray drying, stabilizing emulsion droplets (Vega & Roos, 2006). The questions that arise from the above discussion are: (a) could a very high concentration of protein (thicker interface and excess protein in the continuous phase) form a matrix that is able to protect the emulsion droplets from re-coalescence during spray drying in the absence of MD?; (b) could MD substitute for protein in these emulsions? As the stabilizing abilities of both WPI and NaCas were very similar in these emulsion systems during spray drying, WPI was used to address these questions.

The d_{43} values of parent and redispersed emulsions formed with WPI and containing 1.0% (w/w) MD are shown in Fig. 4.7. The decrease in the d_{43} values of the parent emulsions with an increase in the protein concentration was similar to that observed in emulsions containing 12.0% (w/w) MD, as shown in Fig. 4.5a. However, d_{43} showed a much greater shift towards a larger size range upon spray drying, compared with the corresponding parent emulsions. This shift in particle size was especially amplified at a WPI concentration of 1.0% (w/w), as expected. However, at protein concentrations as high as 10.0% (w/w), the d_{43} values of the

redispersed emulsions were still considerably higher than that of the parent emulsions (Fig. 4.8). When the redispersed emulsions were diluted 1:1 with SDS solution (2.0%, w/w), the droplet size distribution changed very slightly except for the redispersed emulsion containing 1.0% (w/w) WPI. This suggested disruption of the droplet interface during spray drying, causing re-coalescence at all WPI concentrations used. At a protein concentration of 1.0% (w/w), some bridging flocculation may also have occurred, resulting in much larger d_{43} values.

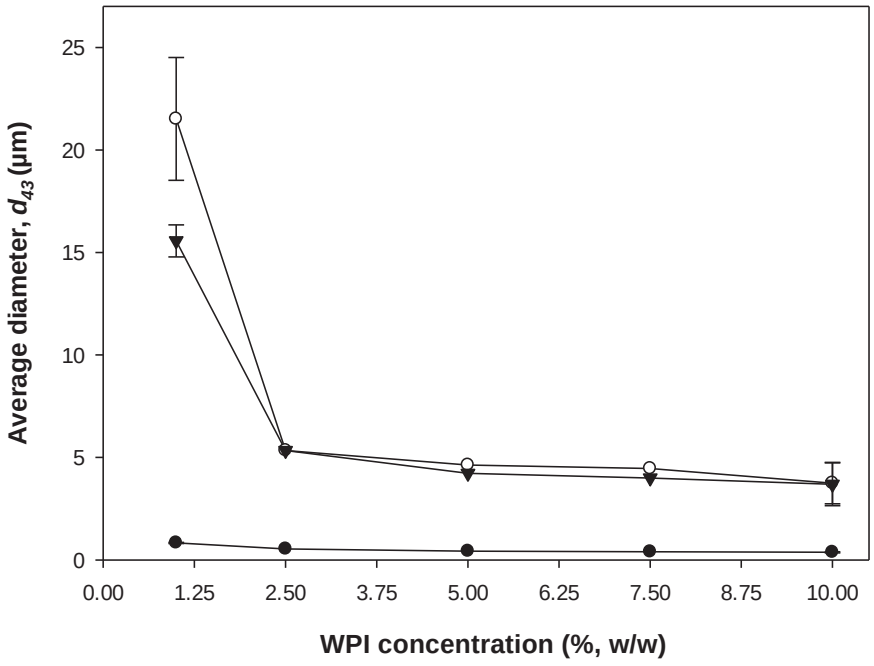


Figure 4.7 *Average droplet diameter (d_{43}) values of parent (●) and redispersed (○) emulsions (1.0% w/w MD, 20.0% w/w soybean oil) as a function of the concentration of WPI (1.0−10.0% w/w). The redispersed emulsions were also diluted (1:1) with 2.0% w/w SDS solution (▼).*

It is expected that a protein concentration of > 2.0% (w/w) in the emulsion would yield a droplet interface that is almost certainly saturated with whey protein and sufficient protein molecules in the continuous phase to stabilize the air/water interface of the drying droplets. However, the shifts in the d_{43} values upon spray drying and redispersion at these concentrations were nearly 10-fold greater than those of the parent emulsions. For instance, at 7.5% protein concentration, the d_{43} value of the redispersed emulsion was 3.9μm as compared its corresponding parent emulsion d_{43} value of 0.4μm. Faldt and Bergenstahl (1996) also reported that the emulsion droplet sizes were preserved upon spray drying in an emulsion series containing both lactose and whey protein in the matrix compared with whey protein only. They concluded that a critical amount of lactose was required to effectively spray dry emulsions.

From the results presented in Fig. 4.4, a protein concentration of 0.5% (w/w) showed a marked shift in the d_{43} values towards larger values upon spray drying and redispersion of the emulsion. Therefore, this concentration of WPI was chosen to examine the effects of the concentration of MD on the redispersion behaviour of whey-protein-stabilized emulsions. The d_{43} values of emulsions (0.5%, w/w WPI, 20.0%, w/w, soybean oil) as a function MD concentration (1.0−25.0%, w/w) are shown in Fig. 4.9. A shift in the d_{43} values towards lower size ranges were observed with an increase in the concentration of MD in the parent emulsions, even at a fixed protein concentration (0.5%, w/w). Upon spray drying and redispersion, the d_{43} values of all emulsions shifted towards a larger size range, compared with the respective parent emulsions, up to 15.0% (w/w) MD. The d_{43} values for the emulsion containing 25.0% (w/w) MD showed some aggregation/flocculation.

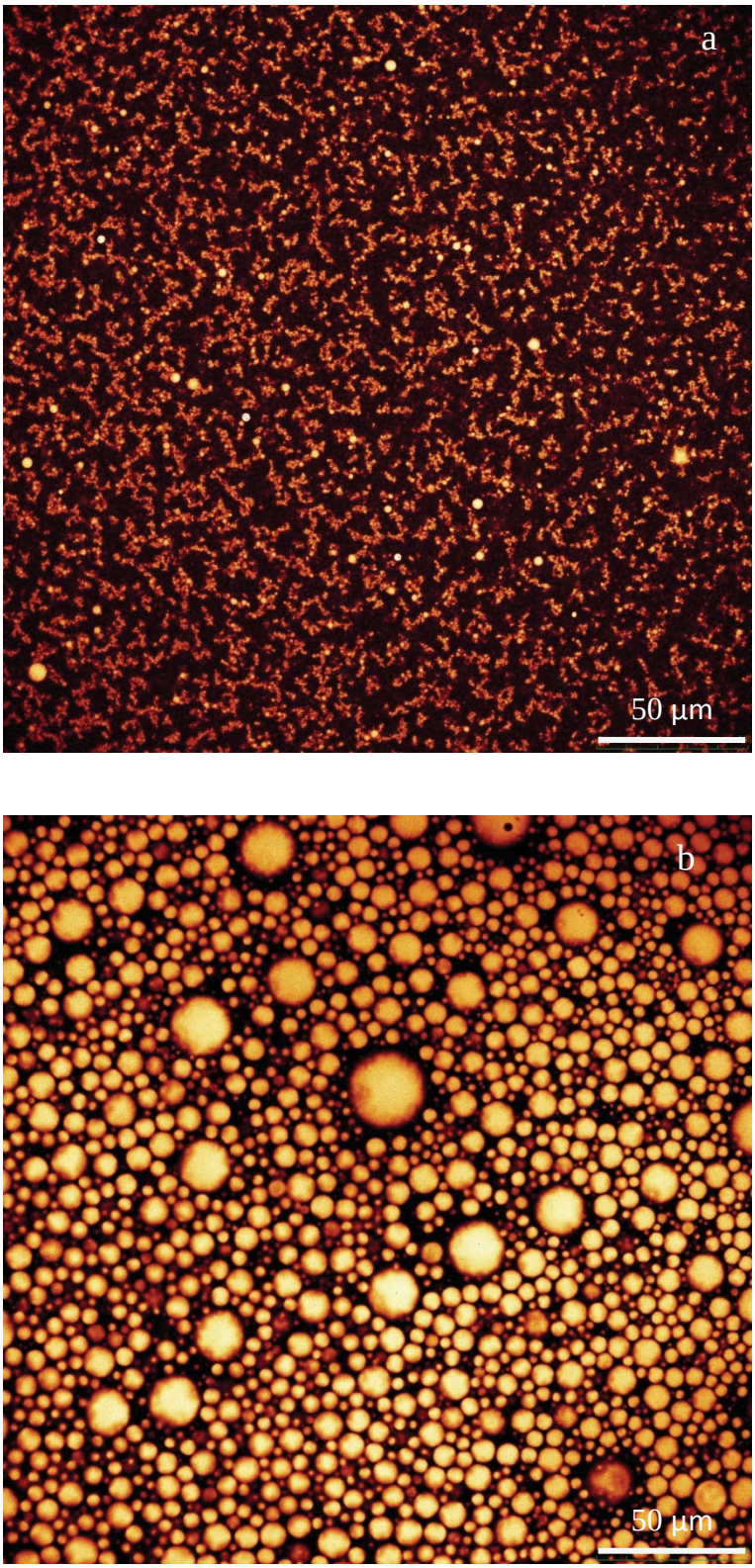


Figure 4.8 *Confocal micrographs of emulsions (10.0% w/w WPI, 20.0% w/w soybean oil, 1.0% w/w MD) before (a) and after (b) spray drying.*

The increase in the d_{43} values in the redispersed emulsions (diluted with 2.0% SDS solution) containing 20.0% and 25.0% (w/w) MD (1.2μm and 0.9 μm respectively) as compared to their corresponding parent emulsions (0.6μm and 0.5μm respectively) was approximately 2-fold, compared with a 10-fold increase the redispersed emulsions (diluted with 2.0% SDS solution) containing high concentrations of WPI (7.5 and 10.0%, w/w) at a low concentration of MD (1.0%, w/w) (Fig. 4.7). The comparison of parent and redispersed emulsions diluted with SDS indicated the real shift in the average droplet diameter values due re-coalescence and discounts the shift caused due to aggregation or flocculation.

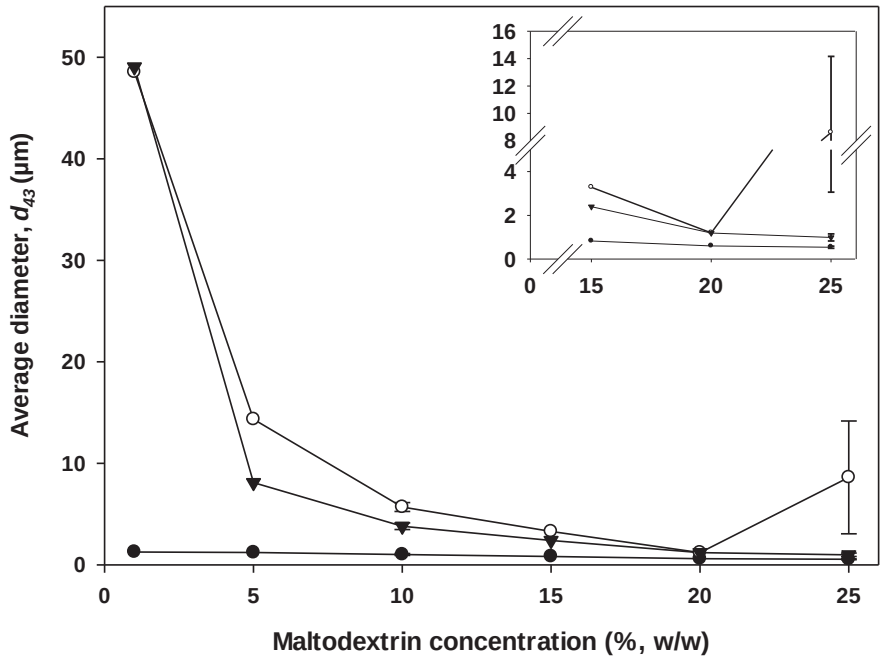


Figure 4.9 *Average droplet diameter (d_{43}) values of emulsions (0.5% w/w WPI, 20.0% w/w soybean oil) as a function of the concentration of MD before spray drying (●), after redispersion of the powder in water (○) and after redispersion in water and 2.0% SDS solution (▼). MD was added to the parent emulsion prior to microfluidization.*

It was expected that, with an increase in the MD concentration, the apparent viscosity of the emulsion would also increase, which would result in a decrease in the average droplet diameter during emulsion formation. This is because of the increase in the drainage time of liquid between colliding droplets during emulsification, which reduces the probability of coalescence. Therefore, a larger surface area is available for adsorption of the protein molecules (Tesch & Schubert, 2002). This effect is indeed visible in Fig. 4.9.

Subsequently, another set of emulsions with 0.5% (w/w) WPI was prepared, in which the MD powder (1.0−25.0%, w/w) was mixed (using a magnetic stirrer) into the emulsions after the homogenization step, to ensure that the increased MD concentration and the apparent viscosity of the emulsion had no effect on the d_{43} values of the homogenized parent emulsions. In Fig. 4.10, the parent emulsions show no change in the d_{43} values with an increase in the MD concentration, as expected. The d_{43} values of these emulsions upon spray drying and redispersion showed a shift towards larger values compared with the respective parent emulsions.

When compared with the d_{43} values of the redispersed emulsions (diluted with 2.0% SDS solution) shown in Fig. 4.9, the d_{43} values obtained in these redispersed emulsions (Fig 4.10; diluted with 2.0% SDS solution) were higher at all MD concentrations. However, there was a similar trend in these values to that in Fig. 4.9; that is, a shift towards smaller values was seen with an increase in the MD concentration. The increase in the d_{43} values of the redispersed emulsions (diluted with 2.0% SDS solution) containing 10.0% (w/w) MD (Figs. 4.9 and 4.10) was ~ 4-fold as compared to their parent emulsions regardless of the point of MD addition. At 10.0% (w/w) MD, the d_{43} values of the redispersed emulsions (diluted with 2.0% SDS solution) were 3.8μm and 5.4μm as compared to the d_{43} values of its parent

emulsions of 1μm and 1.3μm when MD was added to the parent emulsion prior to or after emulsification respectively. This increase was comparable with the result presented in Fig. 4.4da. The emulsions containing 1.0−10.0% (w/w) MD were found to contain some aggregated/flocculated droplets because the d_{43} values shifted to a smaller size range upon the addition of SDS solution. The d_{43} value in the redispersed emulsions (diluted with 2.0% SDS solution) containing 20.0 and 25.0% (w/w) MD increased to nearly twice that of the parent emulsions (Figs. 4.9 and 4.10). An overall improvement in the stability of the droplets was observed with an increase in the MD concentration in the emulsion (20.0−25.0%, w/w, 0.5% w/w, WPI).

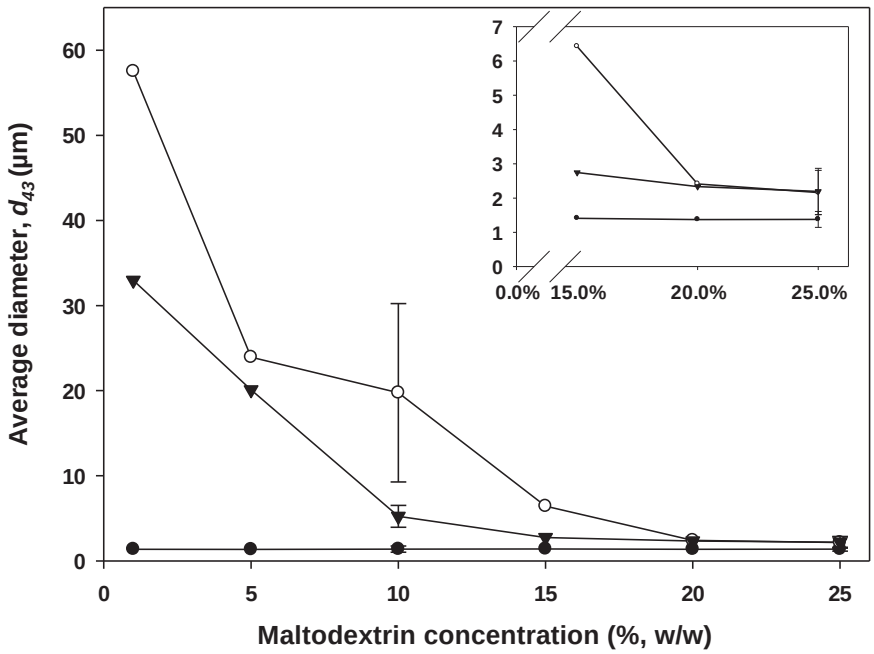


Figure 4.10 *Average droplet diameter (d_{43}) values of emulsions (0.5% w/w WPI, 20.0% w/w soybean oil) as a function of the concentration of MD before spray drying (●), after redispersion of the powder in water (○) and after redispersion in water and 2.0% SDS solution (▼). MD was added to the parent emulsion subsequent to microfluidization.*

Even though well documented, the exact mechanism of the stabilization of proteins by sugars during the drying process is not completely understood (Chang & Pikal, 2009). It is generally believed that the removal of water from the system during spray drying results in some of the hydrogen bonding between the protein molecules and water being disrupted, leading to conformation fluctuations. The presence of a sugar or a polyol during drying provides appropriate hydrogen bonding and polar interactions to maintain the native protein conformation, along with its functionality (Cicerone & Douglas, 2012). In addition, some residual water presides at the surface of the protein molecule in preference to the co-solvent (preferential exclusion), and this interfacial water provides stability to the protein molecule by favouring the folded state over the unfolded state (Cicerone & Douglas, 2012; McClements, 2010).

It is also considered that sugars are capable of making a highly viscous glass phase (sugar glass) around the protein molecule, which restricts its mobility, thus preventing any major conformational changes. Most previous studies have focused on the effects of dehydration on protein, water and co-solvent mixtures. Little information on the behaviour of protein molecules adsorbed at the droplet surface in emulsions during drying is available. It is likely that, during the spray drying of oil-in-water emulsions, adsorbed protein molecules at the oil/water interface migrate from the interface, affecting the stability of the oil droplets against re-coalescence, and that the formation of viscous glass structures by MD slows down or prevents the migration of protein adsorbed at the oil/water interface to the air/water surface of the drying droplet.

4.5 Conclusions

It is clear from these experiments that the addition of MD to the emulsion formulation is crucial for oil droplet stability. At low protein concentration (0.5%, w/w) and very high MD concentration (25.0%, w/w), stresses induced by spray drying were sufficient for interfacial film rupture as some re-coalescence was observed in these emulsions post redispersion. Re-coalescence was largely avoided by an increase in the protein concentration in the emulsion (> 2.0%, w/w). However, excess protein in the continuous phase in the absence of MD did not appear to provide sufficient oil droplet stability during drying. Above a critical concentration (12.0%, w/w), MD appeared to provide stability to the structure of the protein molecules at the interface and adequate rigidity to the matrix, keeping most of the oil droplets apart, even under the highly stressful environmental conditions during drying.

Chapter 5

Influence of protein concentration on the stability of oil-in-water emulsions formed with aggregated milk proteins during spray drying

5.1 Abstract

The ability of aggregated milk protein products (milk protein concentrate or calcium caseinate) to stabilize oil-in water emulsions during the spray drying process is not well understood. The behaviour of oil droplets in oil-in-water emulsions (12.0%, w/w, maltodextrin; 20.0%, w/w, soybean oil) stabilized with either milk protein concentrate (MPC) or calcium caseinate (CaCas) as a function of protein concentration (0.5–5.0%, w/w) before and after spray drying was determined. In comparison with the respective parent emulsions, spray drying and redispersion caused a shift in the droplet size distribution towards larger values for emulsions made using low concentrations (0.5–2.0%, w/w) of MPC or CaCas. However, the droplet size distribution was affected only very slightly by spray drying when the protein concentration was between 3.0 and 5.0% (w/w). The average droplet diameter values of redispersed emulsions containing CaCas were noticeably larger than those of redispersed emulsions containing MPC at all protein concentrations. The unadsorbed protein concentrations in the parent emulsions were comparable for emulsions containing CaCas and MPC, except at a protein concentration of 5.0% (w/w) when emulsions containing MPC had a higher concentration of unadsorbed protein in the bulk phase. The amount of extractable

The contents of this chapter have been published in a peer reviewed journal article and have been adapted with permission. Please refer to page 266.

oil in MPC-containing powders decreased with an increase in the protein concentration in the emulsions prior to spray drying. However, the amount of extractable oil in CaCas-containing powders was nearly twice that in MPC-containing powders even at a protein concentration of 5.0% (w/w) in the emulsion. The results obtained from this study clearly show that aggregated milk proteins are able to provide stability to oil droplets during the spray drying process, albeit a higher concentration of protein is required as compared to non-aggregated protein products (e.g. sodium caseinate and whey protein isolate).

5.2 Introduction

Milk proteins are important components in a number of categories of processed foods because of their excellent nutritional and functional properties. Many different variations of milk protein products are now commercially available for use as food ingredients, e.g. caseins and caseinates, whey protein concentrates (WPCs) and whey protein isolates (WPIs), milk protein concentrates (MPCs) and other specifically designed products for particular applications (Ye, 2011). The structure, conformation and aggregation state of the protein molecules in milk protein products have a considerable impact on their ability to stabilize the oil droplets in oil-in-water emulsions (Euston & Hirst 1999; Faldt & Bergenstahl 1996; Singh & Ye, 2009; Vega & Roos 2006). MPC products containing 56–82% (w/w) protein are processed directly from skim milk by a combination of ultrafiltration and diafiltration. In these products, the whey proteins remain in their native form and the caseins are retained in micellar form, similar to their structures in milk (Dybowska, 2008; Havea, 2006; Singh & Ye, 2009). In contrast, caseinates are manufactured by the acidification of milk to pH 4.6 and separation of the casein curd. Following washing of the curd, the pH is adjusted to 7.0 with either sodium hydroxide or calcium hydroxide, resulting in sodium caseinate (NaCas) or calcium caseinate (CaCas) respectively (Srinivasan et al., 2003).

The exact state of the casein molecules in NaCas solutions is unknown; NaCas solution has been reported to contain a mixture of individual casein molecules and small aggregates with varying casein composition and with sizes around 10–30 nm (Farrer & Lips, 1999; Lucey et al., 2000). CaCas, more aggregated than NaCas, consists of mixtures of casein aggregates of different sizes, many of which are much larger than those found in NaCas solutions (Srinivasan et al., 2003).

NaCas shows excellent emulsifying ability, and very stable emulsions can be made at a relatively low protein-to-oil ratio (about 1:50). A stable emulsion can be defined as one which is able to resist any change in its properties such as droplet size distribution, state of aggregation over the time scale of observation (Singh & Ye, 2009; Vega & Roos, 2006). In these emulsions, the saturation surface protein coverage value is about 2.0–3.0 $mg \cdot m^{-2}$ (Dickinson, 1999; Singh, 2005; Singh & Ye, 2009). In contrast, the emulsifying ability of "aggregated" milk protein products, such as MPC and CaCas, is much lower than that of whey protein or NaCas, under similar homogenization conditions. The surface protein coverage of emulsions formed with these products is much higher, generally in the range 5–20 $mg \cdot m^{-2}$, depending on the protein concentration (Euston & Hirst 1999; Srinivasan et al., 1999). Under similar homogenization conditions, MPC and CaCas emulsions exhibit larger average droplet diameters than NaCas emulsions (Euston & Hirst, 1999). In MPC and CaCas emulsions, bridging flocculation occurs at low protein concentrations, because of the sharing of protein aggregates by adjacent droplets. In addition, the spreading of protein at the droplet surface is limited because the aggregates are held together by calcium bonds and/or colloidal calcium phosphate (Euston & Hirst, 1999; Ye, 2011).

Chapter 4 reported that milk-protein-based emulsions formed with > 2.0% protein (NaCas or whey proteins) remained stable during spray drying, as indicated by the change in the average droplet diameter after spray drying. This corresponded to a critical concentration of ~ > 1.0% unadsorbed protein in the bulk phase of the emulsions. However, below this critical unadsorbed protein concentration in the bulk phase of the emulsions, the average droplet diameter shifted towards a larger size range on spray drying. Although the emulsifying and adsorption properties of

MPC and CaCas in oil-in-water emulsions have been well documented (Euston & Hirst, 1999; Ye, 2011; Ye et al., 2000), their ability to stabilize oil droplets during spray drying has not been reported.

A key parameter in spray-dried powdered emulsions is the amount of free oil on the surface of the powder particles. This free oil on the surface determines the flowability and the wettability of the powder. As rapid and complete reconstitution is a key quality indication for consumers, an understanding of the mechanism of the migration of oil to the surface of the powder particle during drying will be very useful (Kim et al., 2009; Murrieta-Pazos et al., 2012). The objective of this work was to evaluate the stabilizing ability of aggregated milk protein products, i.e. MPC and CaCas, during the spray drying of oil-in-water emulsions and to investigate its impact on the surface composition of the final powder

5.3 Materials and methods

Milk protein concentrate (MPC), calcium caseinate (CaCas), soybean oil and maltodextrin (MD) were used in this study. The details of these materials and the equipment used in the study have been described in Chapter 3 (section 3.1-3.2). All methods used in this study are described in the following sections. The process of emulsion preparation, spray drying and analyses used in this study has been shown is shown in Figure 5.1.

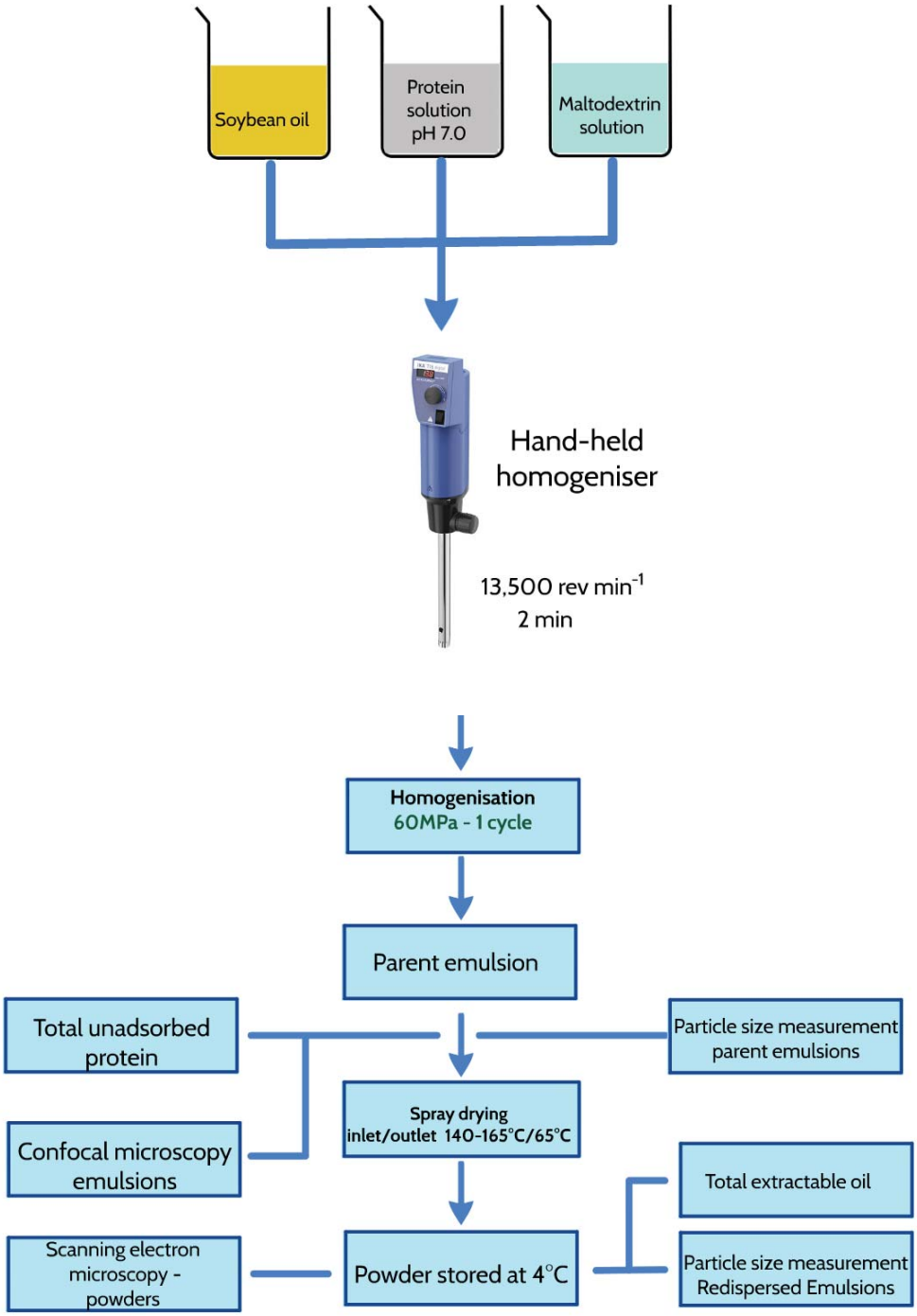


Figure 5.1 *Process flow diagram showing emulsion preparation, spray drying and analyses for this study.*

5.3.1 Emulsion preparation

Proteins (MPC or CaCas) were dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solution was stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solution was then adjusted to 7.0 using 1 M HCl or 1 M NaOH if required. Similarly, MD solution was prepared using the same stirring equipment, with stirring until the powder was dissolved completely. Soybean oil at 20.0% (w/w) was blended with the aqueous solutions of protein and MD using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Approximately, 500 mL of emulsion was prepared for each variation for spray drying purposes.

5.3.2 Spray drying

All emulsions were spray dried using a Buchi bench-top spray drier (B-290, Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current configuration and fitted with a two-fluid nozzle (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The inlet temperature varied between 140 and 165 °C and the outlet temperature was maintained at 65 °C. The resulting powders were collected in air-tight containers, allowed to cool to ambient temperature and then stored at 4 °C for further analysis.

5.3.3 Determination of average droplet diameter

The diameters of the oil droplets for both the parent emulsions (fresh emulsions which have not been spray dried) and the redispersed emulsions (emulsions

prepared by dissolving powder in RO water) were determined by the laser light scattering method as described by Jafari et al. (2007) and Ye (2011) using a Mastersizer E (Malvern Instruments, Malvern, Worcestershire, UK). Redispersed emulsions were prepared by reconstituting the dried powder in RO water to obtain the same total solids content as the parent emulsion. Powder was slowly added to RO water and the dispersions were stirred for at least 30 min before particle size measurements. In some cases, the emulsions were dispersed in 2.0% (w/w) SDS before the measurements. The comparison of the size distributions in RO water alone and with added SDS indicated flocculation/aggregation during emulsification/redispersion (Sanchez & Patino, 2005).

5.3.4 Total unadsorbed protein concentration

The total unadsorbed protein concentration was determined using the method of Ye (2008) with some modifications. Emulsions (30 g) were centrifuged at 45,000 *g* for 45 min at 20 °C in a temperature-controlled ultracentrifuge (Sorvall RC5C, DuPont Co., Wilmington, DE, USA) and the subnatant were carefully removed using a syringe. The cream layer was dispersed in RO water and re-centrifuged at 45,000 *g* for 45 min. Again, the subnatant and the cream layer were collected carefully. Each subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corporation, Billerica, MA, USA). The filtrates were analysed separately for total protein using the Kjeldahl method (1026 Distilling Unit and 1007 Digestor Block, Foss Tecator AB, Höganäs, Sweden). A factor of 6.38 was used to convert nitrogen to protein content. The total unadsorbed protein concentration (%) was calculated by adding the percentage protein concentrations in each of the subnatant collected after centrifugation.

5.3.5 Total extractable oil

The total extractable oil content of the powdered emulsions was determined using solvent (petroleum ether, boiling point 40–60 °C) extraction, as described by Kim et al. (2005). One gram of powder was carefully weighed on to a filter paper (No. 4 Whatman, Maidstone, Kent, UK) and was washed four times with 5 mL of petroleum ether. The mixture of solvent and oil was collected in a conical flask, evaporated over a steam bath and left overnight in the fume hood to allow further evaporation of the remaining ether. The flask was then placed in a 70 °C oven for 1 h and then cooled in a desiccator. The amount of extracted oil was recorded as milligrams of extractable oil per gram of powder.

5.3.6 Confocal scanning laser microscopy

About 500 μL of emulsion (both parent and redispersed) sample was taken in an Eppendorf tube, 10 μL of Nile red (fluorescent dye, 0.1% w/v in acetone) was added and the sample was gently shaken. A volume of 60 μL from this sample was placed on a concave microscope slide. A cover slip was carefully placed and the sample was observed under the microscope. A 63 X oil immersion objective with sequential excitation lines of 488 nm from an argon laser was used.

For powder samples, the fluorescent dyes were dissolved in the oil phase (Nile red, 0.02%, w/w) and the protein solution (Fast green, 0.01%, w/w) followed by emulsion preparation and spray drying (Fig. 4.2). The resulting powder was carefully mounted on to a concave microscope slide using DPX (non-aqueous mounting medium). A cover slip was placed carefully on top, avoiding air bubbles. A 100 X oil immersion objective with sequential excitation lines of 488 nm from an

argon laser and of 633 nm from a helium−neon laser was used. All images were acquired at 20 °C (± 2 °C).

5.3.7 Scanning electron microscopy

The powder samples were placed on the scanning electron microscopy stubs using two-sided adhesive tape (Scotch, 3M NZ, Auckland, New Zealand). The specimens were subsequently coated with gold using a sputter coater (Balzers SCD 050 Sputter Coater, Balzers, Liechtenstein). The coated samples were then analysed using the microscope operating at an accelerating voltage of 20 kV. Micrographs, representing the microstructure of the powdered emulsions, were obtained using the FEI Quanta software installed on the PC connected to the microscope.

5.3.8 Data analysis

Each data point represents average value of two determinations on two separate emulsions (measured in duplicates). Error bars in graphs represent standard deviation of average values of two determinations on separate emulsions. All graphs were drawn using Sigmaplot 13 graphing and data analysis software (Systat Software Inc., San Jose, CA, USA).

5.4 Results

5.4.1 Droplet size distributions

The droplet size distributions of emulsions (12.0%, w/w, maltodextrin; 20.0%, w/w, soybean oil) containing MPC or CaCas at concentrations from 0.5 to 5.0% (w/w) before and after spray drying are shown in Figs. 5.2 and 5.3 respectively. The size distributions were monomodal for all parent emulsions (before drying) containing MPC and shifted towards a smaller size range as the concentration of MPC in the

bulk phase was increased from 0.5 to 2.0% (w/w). There was no noticeable change in the distribution when the concentration of MPC was increased further from 2.0 to 5.0% (w/w, Fig. 5.2).

When the MPC emulsions were spray dried and then redispersed in RO water, for emulsions containing ≤ 2.0% (w/w) MPC, the size distributions shifted towards a larger size range compared with the parent emulsion at the corresponding protein concentration (Fig. 5.2). However, emulsions containing ≥ 3.0% (w/w) MPC showed good stability during spray drying because their size distributions were very similar before and after spray drying (Fig. 5.2).

The parent emulsions prepared using CaCas had bimodal size distributions that were noticeably broader than those of the parent MPC emulsions. As the CaCas concentration in the emulsion was increased from 0.5 to 5.0% (w/w), the size distribution shifted towards a smaller size range (Fig. 5.3). After spray drying, all the redispersed emulsions made with CaCas had multimodal distributions that were shifted noticeably towards a larger size range because of the spray drying (Fig. 5.3). This shift towards a larger size range was greatest at ≤ 1.0% (w/w) CaCas in the emulsion. From these size distribution profiles, only the emulsion prepared with 5.0% (w/w) CaCas showed good redispersion, i.e. no noticeable change in size distribution after spray drying.

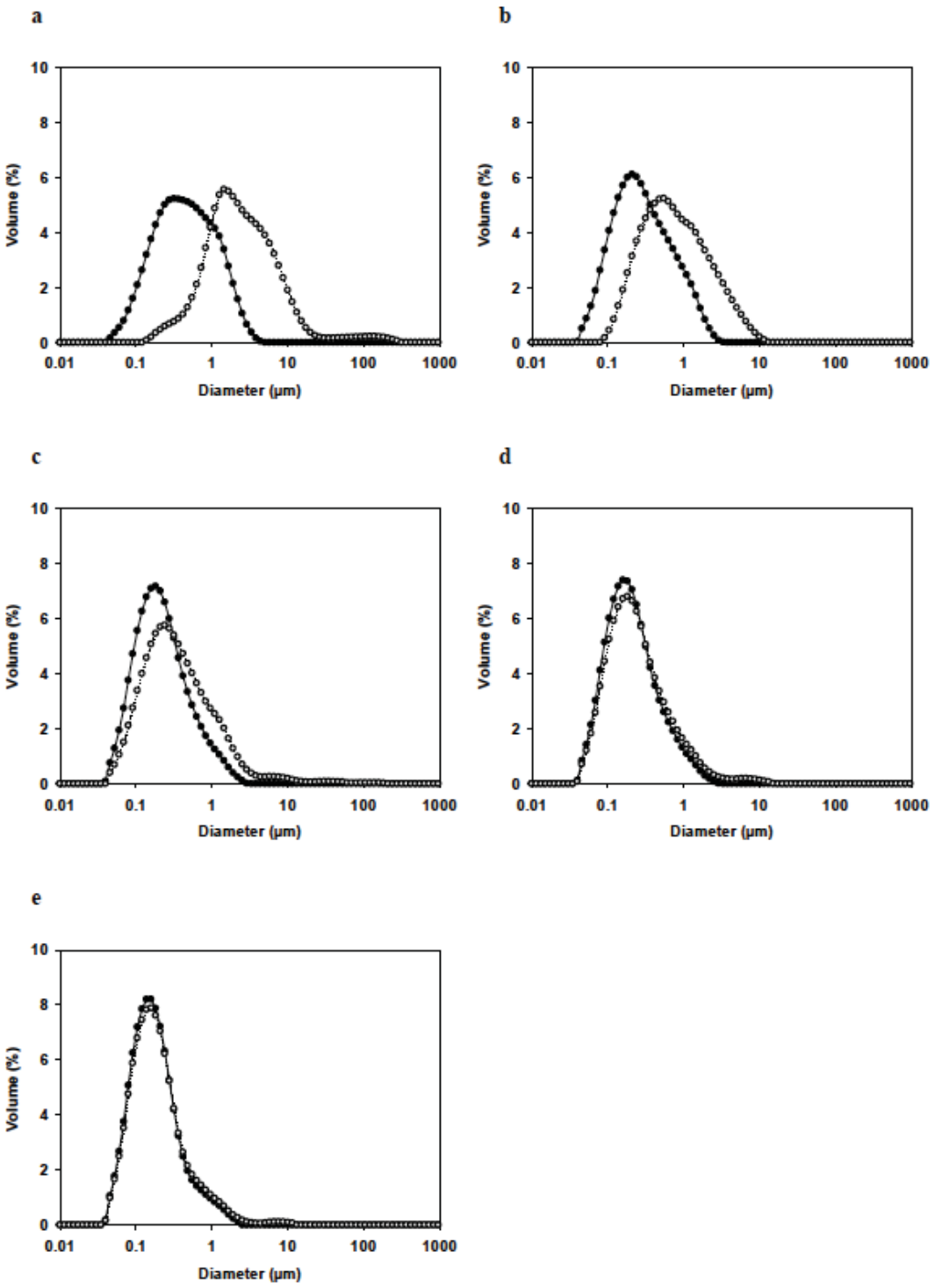


Figure 5.2 *Droplet size distributions of emulsions (0.5−5.0%, w/w, milk protein concentrate, 20.0%, w/w, soybean oil, 12.0%, w/w, maltodextrin) before spray drying (●) and after redispersion (○): a, 0.5%; b, 1.0%; c, 2.0%; d, 3.0%; e, 5.0% protein.*

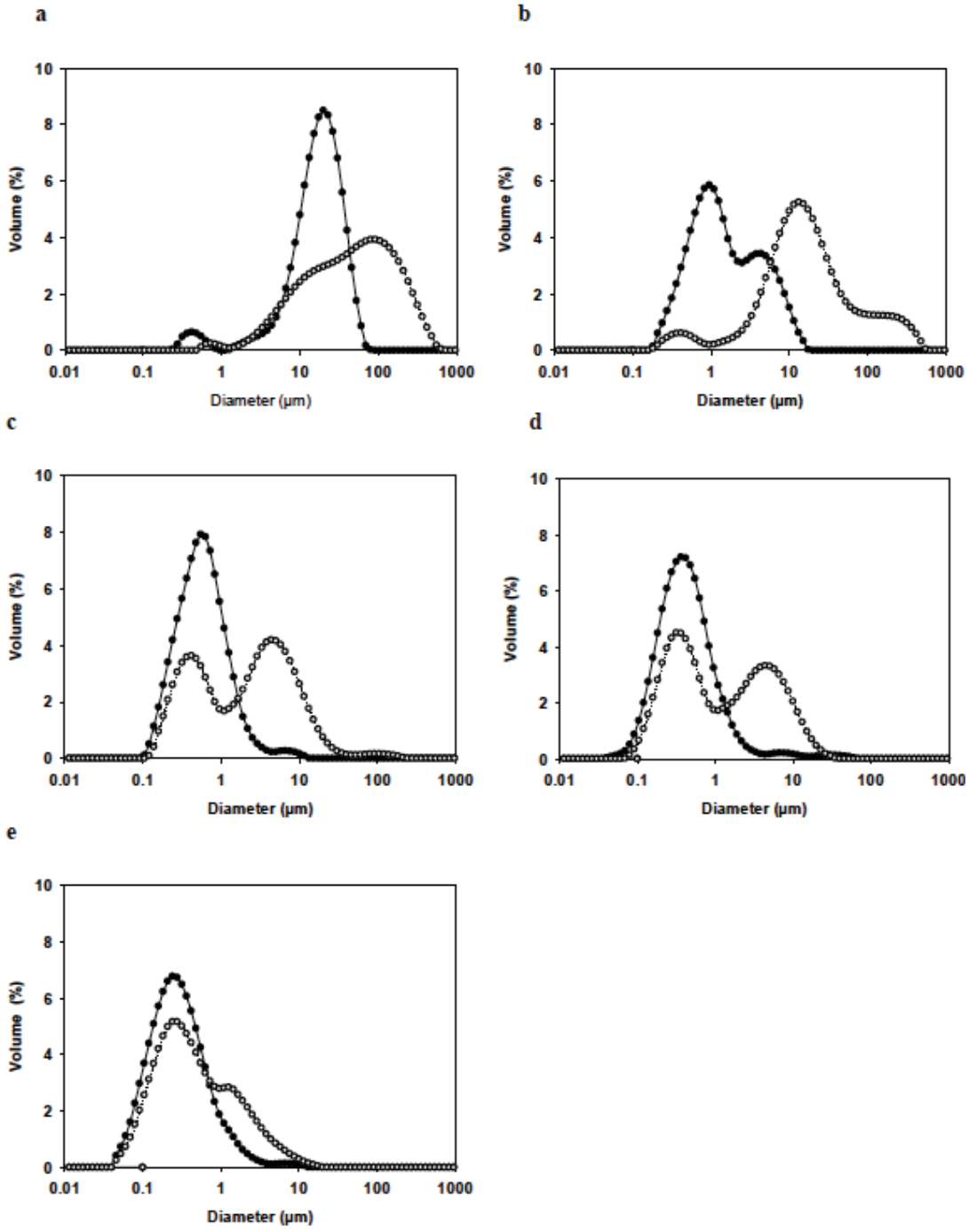


Figure 5.3 *Droplet size distributions of emulsions (0.5−5.0%, w/w, calcium caseinate, 20.0%, w/w, soybean oil, 12.0%, w/w, maltodextrin) before spray drying (●) and after redispersion (○): a, 0.5%; b, 1.0%; c, 2.0%; d, 3.0%; e, 5.0% protein.*

The changes in average droplet size (d_{43}) of the MPC and CaCas emulsions upon spray drying are shown in Fig. 5.4. The d_{43} values of the redispersed emulsions made with ≤ 1.0% (w/w) MPC or CaCas were considerably higher than those of the corresponding parent emulsions. When these emulsions were diluted (1:1) with 2.0% (w/w) SDS solution (which breaks hydrophobic interactions and displaces protein molecules from the oil–water interface), there was a significant reduction in d_{43}, which indicated that the spray drying had caused flocculation and re-coalescence of the emulsion droplets. This was also visually evident in the confocal micrographs of the emulsion containing 0.5% (w/w) CaCas, as shown in Fig. 5.5. According to the micrographs, droplet re-coalescence seemed to be the dominating phenomenon. There was no change in the d_{43} values when the redispersed emulsions made with 5 % (w/w) MPC or CaCas were diluted with 2.0% (w/w) SDS solution (Fig. 5.4).

It should be noted that the d_{43} values of the redispersed emulsions containing CaCas were more than three times larger than those of the redispersed emulsions containing MPC at any given protein concentration. For example, at 1.0% (w/w) protein in the emulsions, the d_{43} values of the redispersed emulsions containing MPC and CaCas were approximately 1 μm and approximately 20 μm respectively.

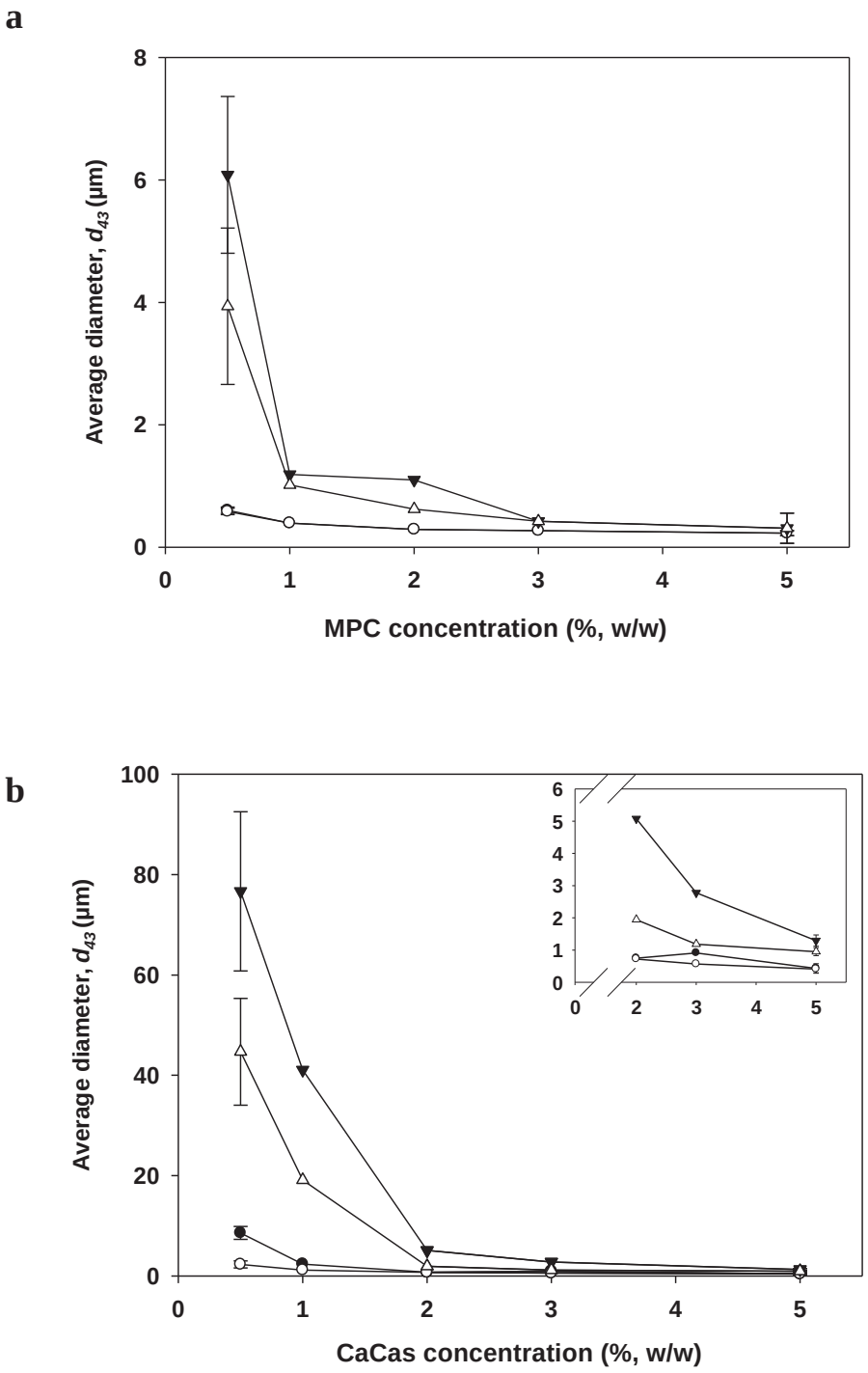
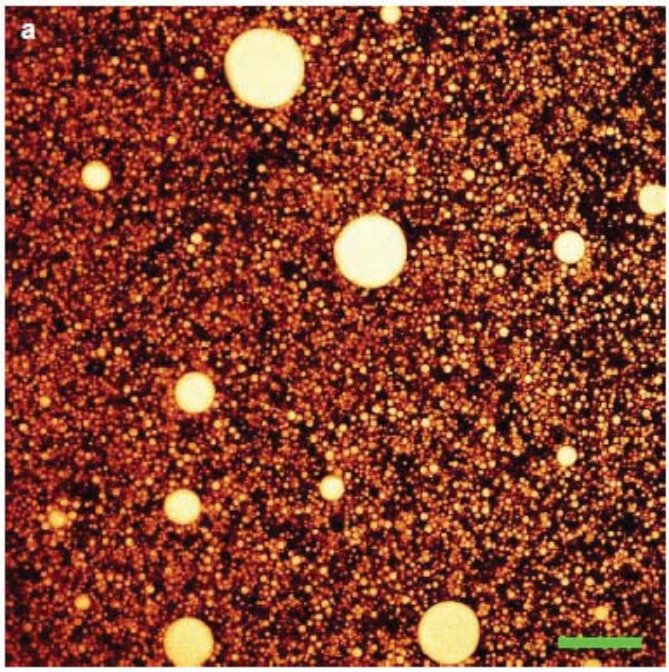


Figure 5.4 *Average droplet diameter (d_{43}) values of parent (●) and redispersed (▼) emulsions containing 20.0% (w/w) soybean oil, 12.0% (w/w) maltodextrin and 0.5−5.0% (w/w) milk protein concentrate (MPC) (a) or calcium caseinate (CaCas) (b). The parent (○) and redispersed (Δ) emulsions were also diluted (1:1) with 2.0% (w/w) sodium dodecyl sulphate solution.*

a



b

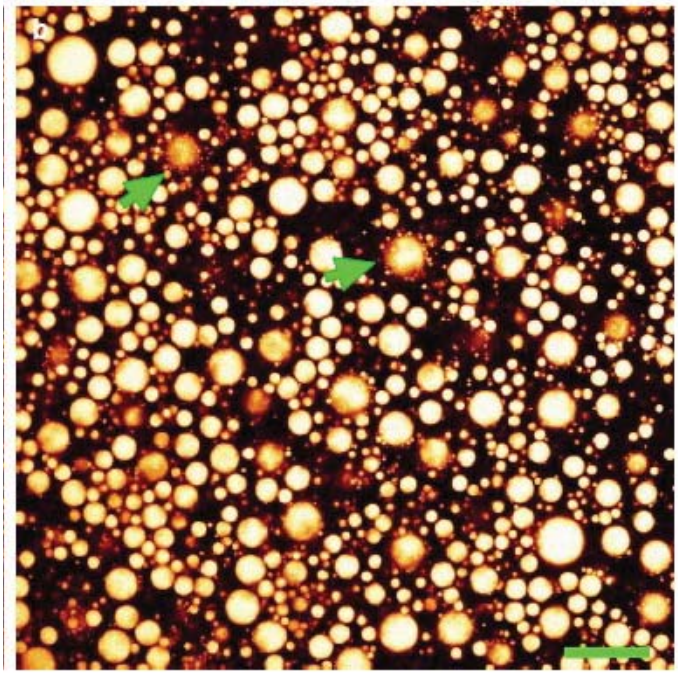


Figure 5.5 *Confocal micrographs of emulsions (0.5%, w/w, calcium caseinate, 20.0%, w/w, soybean oil, 12.0%, w/w, maltodextrin) stained with Nile blue: a, parent emulsion; b, redispersed emulsion after spray drying. The scale on the micrographs represents 50 µm. Green arrows illustrate bridging flocculation (b).*

5.4.2 Total unadsorbed protein concentration

In Chapter 4 it was suggested that the total unadsorbed protein in the bulk phase plays a crucial role in the stability of the oil droplets during spray drying. Therefore, the total concentration of unadsorbed protein in the bulk phase of the parent emulsions stabilized by both MPC and CaCas was determined. The amounts of unadsorbed protein in the bulk phase of the parent emulsions made using MPC and CaCas were comparable each other at protein concentrations between 0.5% to 3.0% (w/w) (Fig. 5.5). However, at 5.0% protein concentrations in emulsions, the amount of protein that was free in the bulk phase were higher for the MPC-stabilized emulsion (3.5%) as compared to CaCas-stabilized emulsion (2.25%).

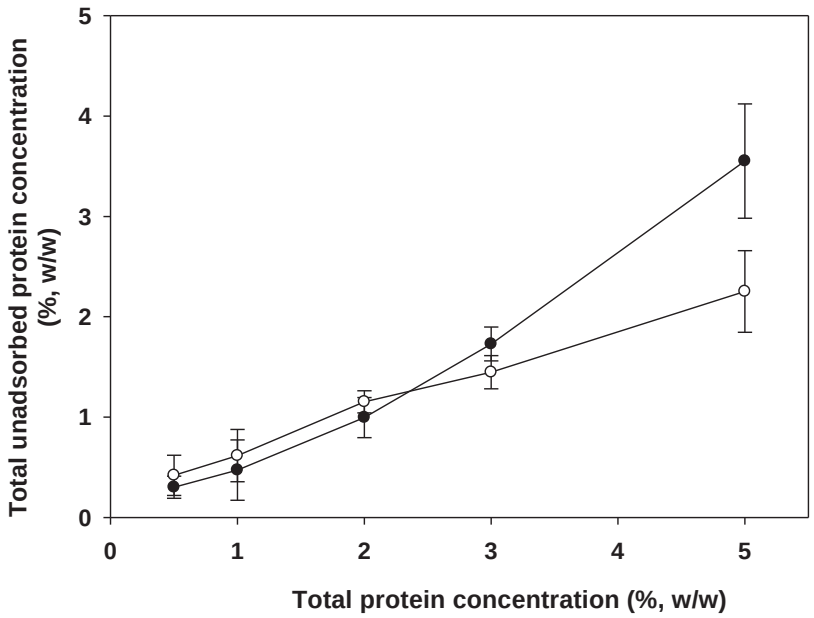


Figure 5.6 *Total unadsorbed protein concentration (%, w/w) in the bulk phase of the parent emulsions containing either milk protein concentrate (●) or calcium caseinate (○) as a function of the total protein concentration (%, w/w).*

5.4.3 Total extractable oil

The extractable oil contents ($mg \cdot g^{-1}$) of the spray-dried emulsions stabilized with MPC and CaCas are shown in Fig. 5.7 as a function of protein concentration (0.5–5.0%, w/w). For the emulsions stabilized with MPC, the amount of extractable oil decreased as the MPC concentration in the powder was increased up 3.0% (w/w), with no further change between 3.0 and 5.0% (w/w) MPC. There was a similar trend for the emulsions stabilized with CaCas; however, the amounts of extractable oil were much higher in the CaCas-containing powders than in the MPC-containing powders. For both emulsions, the greatest amount of extractable oil was observed at the lowest protein concentration, i.e. 0.5% (w/w).

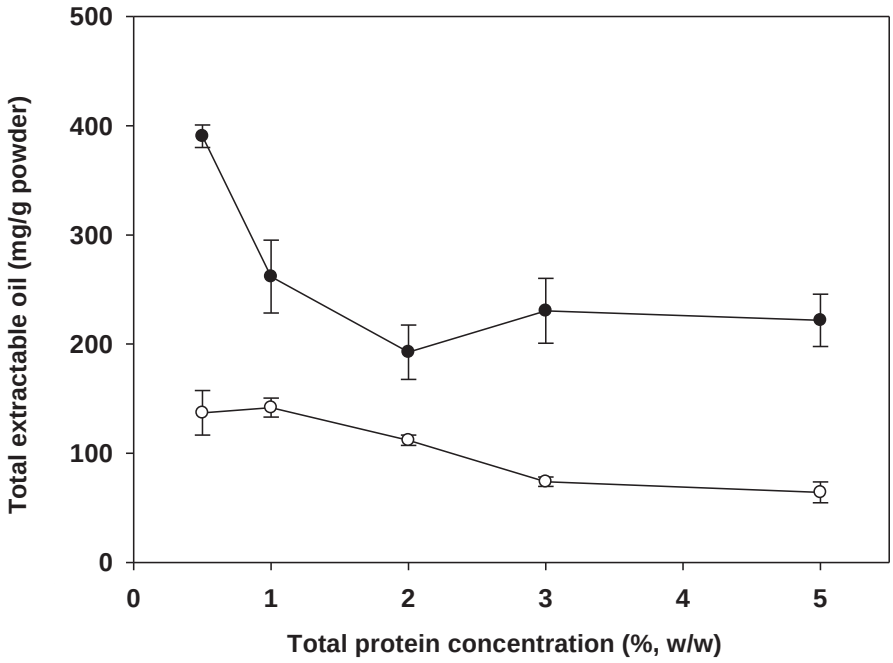


Figure 5.7. *Total extractable oil (mg/g of powder) for powders containing either milk protein concentrate (○) or calcium caseinate (●) as a function of protein concentration in the emulsions used to make the powders.*

5.4.4 Microstructure of spray-dried powder particles

The electron micrographs of dried emulsions containing high and low concentrations of MPC and CaCas showed considerable differences in powder particle structure. At low MPC and CaCas concentrations, the powder particles appeared to be excessively agglomerated, almost giving a “molten” appearance, which possibly indicated high amounts of free oil on the surfaces of the powder particles (Figs. 5.8a and 5.8b). At high MPC and CaCas concentrations, the powder particles were observed to be mostly well separated and had considerable indentations. This appearance of dents or a shrivelled texture was indicative of the formation of vapour bubbles inside the particles because of differences in the local vapour pressure. It has been suggested that, when powder particles reach parts of the drier in which the temperatures are relatively cooler, e.g. cyclones, the particles tend to deflate, resulting in a shrivelled appearance (Nijdam & Langrish, 2006).

The distribution of oil droplets within the powder particles containing MPC was also studied using confocal scanning laser microscopy. The micrographs showed the presence of coalesced oil droplets (in red) in powdered emulsions containing 0.5% (w/w) MPC (Fig. 5.9a). In contrast, there were evenly distributed oil droplets inside the matrix of powder particles in the powdered emulsions containing 5.0% (w/w) MPC (Fig. 5.9b). An over-representation of protein (green colour) on the surface of the powder particles at both MPC concentrations of 0.5 and 5.0% (w/w) in powders was also observed (Fig 5.9).

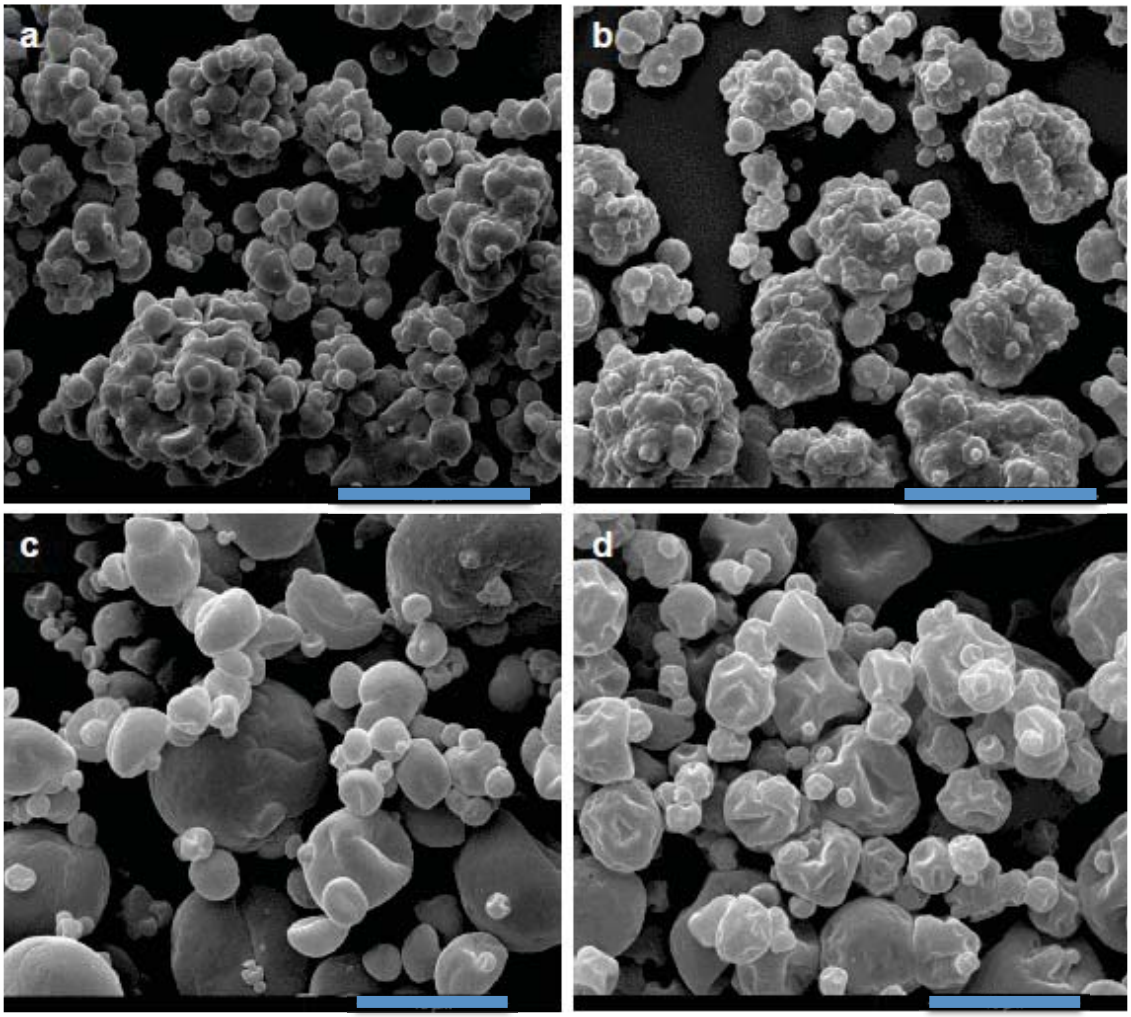


Figure 5.8 *Electron micrographs of emulsion powder particles (20.0%, w/w, soybean oil, 12.0%, w/w, maltodextrin): a, 0.5% (w/w) milk protein concentrate; b, 0.5% (w/w) calcium caseinate; c, 5.0% (w/w) milk protein concentrate; d, 5.0% (w/w) calcium caseinate. Scale is 50μm (a, b) and 10μm (c, d).*

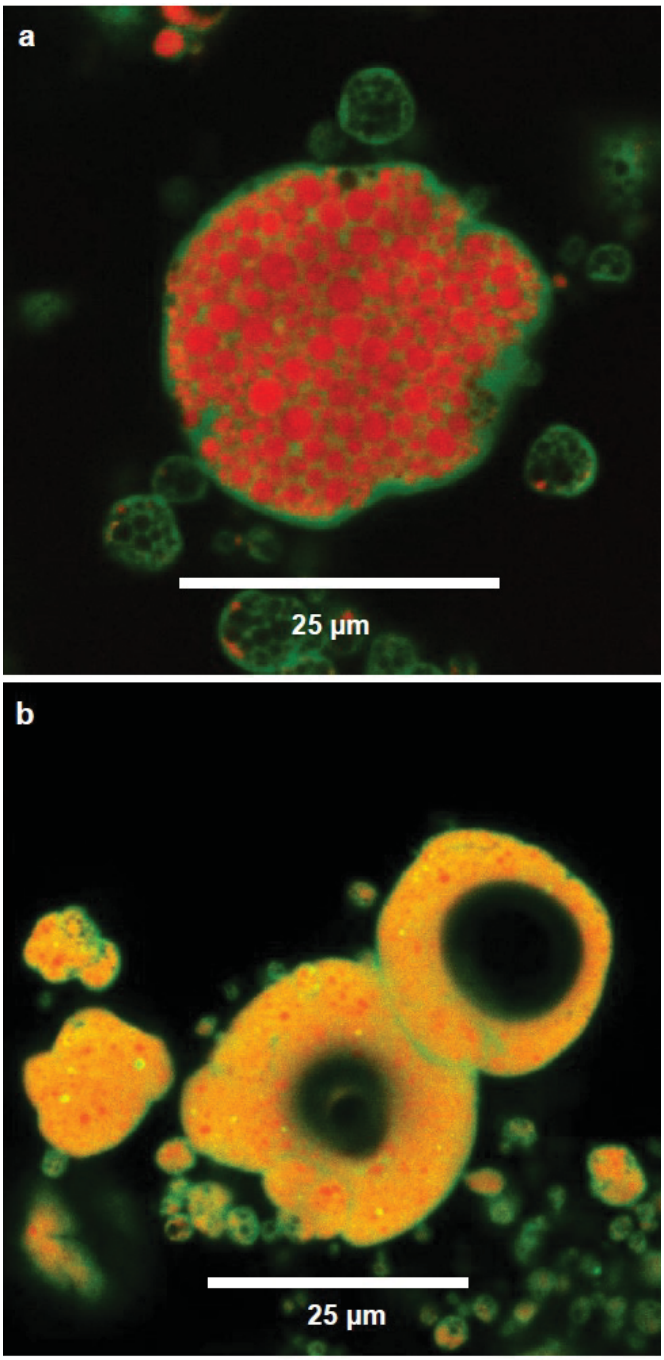


Figure 5.9 *Confocal micrographs of emulsion powder particles (20.0%, w/w, soybean oil, 12.0%, w/w, maltodextrin) stained with Nile red and fast green: a, 0.5% (w/w) milk protein concentrate; b, 5.0% (w/w) milk protein concentrate. Red (and orange) represents the oil phase and green represents proteins. The black hole inside the powder particle is an air vacuole. Scale as shown on the micrographs.*

5.5 Discussion

At a given protein concentration, the emulsifying ability of a protein was determined by the average droplet diameter of the droplets generated under defined homogenization conditions. Smaller droplet sizes are indicative of superior emulsifying ability (Euston & Hirst, 1999). Milk proteins, either as individual molecules or as aggregated species, rapidly adsorb on to the surface of the oil droplets during emulsification, providing stability to the droplet. A number of factors affect the adsorption of protein on to the oil–water interface during homogenization, including protein concentration, oil volume and the state of protein aggregation (Singh & Ye, 2009).

There are some important differences between MPC and CaCas in terms of the aggregation state and the composition of the proteins that are likely to influence their ability to form and stabilize oil-in-water emulsions. MPC contains both whey proteins and caseins, which are largely in the form of casein micelles (Euston & Hirst, 1999; Kelly, 2011). In addition, a small proportion of the protein material remains insoluble in water and consists mainly of large protein aggregates that are held together by non-covalent linkages. The majority of these insoluble aggregates have been reported to be composed of caseins and minor whey proteins (Havea, 2006). In contrast, CaCas consists of various casein aggregates ranging in size from 20 to 1000 nm (Srinivasan et al., 2001). About 25% of the casein in a CaCas solution appears to be highly aggregated and these large aggregates are generated mainly via interactions of calcium with α_{s1}- and α_{s2}-caseins (Euston & Hirst, 1999; Srinivasan et al., 1999).

Because of the presence of whey proteins in addition to casein micelles, the emulsions formed with MPC had narrower size distributions at all protein concentrations than the emulsions formed with CaCas. The casein aggregates in CaCas also have the ability to adsorb at the droplet surface, but not as efficiently as the monomeric caseins or the whey proteins. This would have a signification effect, especially at low protein concentrations, promoting re-coalescence and bridging flocculation, as the aggregates in CaCas would reduce the emulsifying ability by resisting rearrangement and spreading on the surface of the oil droplet, when compared with MPC-containing emulsions (Euston & Hirst 1999; Ye, 2011; Figs. 5.2–5.4).

The total unadsorbed protein contents were comparable for the MPC and CaCas emulsions at protein concentrations between 0.5% and 3.0% (w/w). However, at 5.0% protein concentration, the MPC-containing emulsion had a higher unadsorbed protein concentration (Fig. 5.6). However, the protein concentration on the surface of the oil droplets would possibly be higher in the CaCas-containing emulsion than in the MPC-containing emulsion, because of the preferential adsorption of aggregated casein material at the surface of the oil droplets in CaCas-containing emulsions and the larger overall average size of these casein aggregates compared with the aggregated species in MPC-containing emulsions (Srinivasan et al., 2001; Ye, 2011).

In Chapter 4, emulsions made using NaCas and WPI showed overlapping distributions of the parent and redispersed emulsions at protein concentrations above 2.0% (w/w). Below this concentration, the droplet size distribution showed a shift towards a larger size range in both types of emulsion. The MPC- and CaCas-containing emulsions showed similar redispersion behaviour, although the average

droplet diameters in the MPC and CaCas emulsions were larger than those in the WPI and NaCas emulsions at a given protein concentration (Figs. 5.2–5.4). In addition, the minimum concentration of protein that was required to produce emulsions that were stable during the spray drying process was 3.0% (w/w), compared with 2.0% (w/w) for the NaCas- and WPI-containing emulsions (Chapter 4). It was suggested that the amount of unadsorbed protein in the bulk phase of the parent emulsions might play a crucial role in stabilizing the oil droplets during spray drying. Protein molecules, because of their high surface activity, are likely to migrate to the newly created air–water interface during spray drying (Vega & Roos, 2006). When the surface of the oil droplet is saturated with protein molecules and the bulk phase of the emulsion has sufficient unadsorbed protein, the oil droplet is stable during drying. However, for emulsions with a low concentration of unadsorbed protein in the bulk phase ($\leq$ 1.0% for WPI or NaCas emulsions), protein molecules could potentially migrate from the surface of the oil droplet to the air–water interface, causing "gaps" in the oil droplet interface and leading to coalescence and/or bridging flocculation (Chapter 4). The concentration of non-aggregated unadsorbed protein molecules would effectively be lower in emulsions made using MPC and CaCas, because of the presence of aggregated species. In addition, casein aggregates are thought to be less surface-active than non-aggregated caseins (Ye et al., 2000). Therefore, a higher concentration of protein in the bulk phase would be required in MPC and CaCas emulsions to stabilize the oil droplets during drying, compared with NaCas and WPI emulsions, as seen in this study.

Even though the emulsions made using MPC and CaCas showed good redispersion behaviour above a protein concentration of 3.0% (w/w), in general, the CaCas-

containing emulsions had higher average droplet diameters than the MPC-containing emulsions at similar protein concentrations (Figs. 5.2–5.4). This was possibly due to the larger aggregated species on the surface of the emulsions containing CaCas compared with the relatively smaller sized aggregated species and the non-aggregated species in the emulsions containing MPC. These larger aggregated species are likely to respond relatively more slowly to redistribution of the adsorbed layer during spray drying because of their comparatively lower hydrophobicity and flexibility, resulting in re-coalescence (Vega & Roos, 2006; Vega et al., 2007).

The focus of this study was on the importance of droplet stability during spray drying, as it is essential that powdered emulsions reconstitute to the same droplet size distribution as in the parent emulsions. The presence of free oil on the surface of powder renders the powder particles hydrophobic, drastically reducing their solubility in water (Nijdam & Langrish, 2006). In addition, it has been shown that oil on the surface allows the formation of weak bridges between particles, leading to a loss of quality through caking. The phenomenon of caking involves the transformation of a free-flowing powder into lumps and ultimately into an agglomerated solid, leading to a loss of functionality, i.e. wettability, flowability, oxidative stability etc. (Vega & Roos, 2006).

The solvent extraction method recovers both oil on the surface and some oil from inside the powder through cracks and pores (Vega & Roos, 2006). Therefore, the extractable oil value potentially reflects the oil-retaining capabilities of the powdered emulsions as well as the presence of oil on the surface. The oil on the surface is thought to originate from the emulsion droplets entering the air–water interface and opening at the surface during drying while water is being removed

from the drying droplet (Hotrum et al., 2002). However, if sufficient protein were available in the bulk phase of the emulsion during drying, the opening of the emulsion droplets would be limited, as proteins would dominate the surface. The decrease in the amount of extractable oil for the MPC-containing emulsions was possibly indicative of this behaviour (Fig. 5.6). At MPC concentrations of 3.0 and 5.0% (w/w) in the emulsions, as the extractable oil value was below 100 $mg \cdot g^{-1}$, less agglomeration was seen in the electron micrographs (Fig. 5.7). In contrast, at lower protein concentrations in the emulsion, the powder particles were highly agglomerated and had higher extractable oil values. At all protein concentrations, the CaCas-containing powdered emulsions had higher levels of extractable oil than the MPC-containing powdered emulsions. At 5.0% (w/w) protein in the emulsion, the electron micrographs did not show “molten” agglomerated particles in the powdered emulsions containing CaCas even though the extractable oil at this concentration was much greater than that in the powdered emulsions made using 0.5% (w/w) MPC, which showed extensive agglomeration. It seems possible that the powdered emulsions made with CaCas were of a highly porous nature and were more pervious to solvent than the powdered emulsions made with MPC.

5.6 Conclusions

Based on this study, it was clear that aggregated proteins were capable of stabilizing oil droplets during the drying process. However, the concentration required of these proteins in emulsions was higher as compared with non-aggregated proteins products such as WPI or NaCas. Above a concentration of 3.0% in emulsions, both MPC and CaCas containing emulsions were stable against stresses induced during the drying process. The emulsion stabilizing properties of

MPC were better than CaCas as the average droplet diameter of the redispersed emulsions showed lower size ranges for MPC containing emulsions when compared with CaCas containing emulsions. The morphologies of the two powders were also observed to be different as CaCas powders showed higher total extractable oil content when compared with MPC containing powder due to CaCas powders possibly being of a highly porous nature and thus unable to retain oil inside the powder matrix.

Chapter 6

The impact of heat treatment on the stability of whey-protein-based oil-in-water emulsions during spray drying

6.1 Abstract

The effects of heat treatment on the behaviour of oil droplets in whey-protein-stabilized oil-in-water emulsions (20.0%, w/w, soybean oil; 12%, w/w, maltodextrin; pH 7.0) during spray drying were studied. Emulsions containing whey protein isolate (WPI, 0.25–5.0%, w/w) were subjected to preheating between 65 and 90ºC as a function of time, cooled to room temperature and then spray dried (outlet temperature 65ºC). The changes in the average droplet diameter (d_{43}) before and after spray drying were examined using laser light scattering. The aggregation of whey proteins as a consequence of preheating was studied using polyacrylamide gel electrophoresis and transmission electron microscopy. These emulsions were also compared with emulsions that were prepared using preheated WPI solutions under similar preheating conditions. All redispersed emulsions that were preheat treated above 70ºC showed a shift in d_{43} towards the larger size range because of droplet re-coalescence as a result of spray drying. Preheating of the whey proteins probably resulted in protein aggregation in both sets of emulsions, which adversely affected their ability to stabilize the emulsion droplets during spray drying. Based on these results, it can be concluded that, during spray drying, the role of

monomeric whey proteins is crucial and that a reduction in the non-adsorbed monomeric whey proteins in the continuous phase leads to oil droplet coalescence.

6.2 Introduction

Because of their excellent functional properties and nutritional quality, whey protein ingredients, such as whey protein isolate (WPI) and whey protein concentrate (WPC), are continually being developed for use in a wide range of food applications. The main proteins present in whey are β-lactoglobulin (β-lg, 50%), α-lactalbumin (α-la, 20%) and bovine serum albumin (BSA, 5%) (Havea, Singh, Creamer, & Campanella, 1998). The emulsion-stabilizing properties of both β-lg and α-la are well documented (Ye, 2008). To improve shelf life and to minimize packaging and shipping costs, emulsions are often spray dried into a powdered format. The main objective of the drying process is to obtain a product that, after redispersion, resembles the original emulsion (Sliwinski, Roubos, Zoet, van Boekel, & Wouters, 2003). However, because of re-coalescence during drying, an increase in the average droplet diameter (d_{43}) upon redispersion of the powder in water has been reported for whey-protein-stabilized oil-in-water emulsions (Hogan, McNamee, O'Riordan, & O'Sullivan, 2001; Jafari, Assaidpoor, Bhandari, & He, 2008). In Chapter 4, compared with the parent emulsions, the size distribution of emulsions stabilized with whey proteins shifted towards a larger size range upon redispersion at a WPI concentration in the emulsions of below 2.0% (w/w). This shift was thought to be due to insufficient unadsorbed whey protein (< ~ 1.0%, w/w) in the aqueous phase of the emulsion during drying. In contrast, optimum stabilization of the emulsion droplets during spray drying was achieved when the amount of unadsorbed whey protein in the bulk phase was higher than this critical value of 1.0% (w/w) (Chapter 4).

For food safety purposes, thermal processing is essential in the manufacture of powdered food products. However, it is well known that β-lg and α-la unfold (denature) from a globular configuration and aggregate upon heat treatment at above 70°C (Euston, Finnigan, & Hirst, 2000). This has a marked impact on their ability to form stable emulsions and provide stability against oil droplet coalescence (Sliwinski et al., 2003). The main factors affecting the size and the structure of the aggregates are the concentration of whey proteins, the heating protocol used, the pH and the concentration of salt (Sliwinski et al., 2003; Nicolai, Britten & Schmitt, 2011). Although the influence of heating over a range of variables in oil-in-water emulsions stabilized by whey protein has been reported (Ye, 2008), very few studies have looked at the impact of preheat treatment on the stability of oil droplets in WPI-stabilized oil-in-water emulsions during drying. The objective of this study was to explore the effects of heating on the stability of oil droplets during spray drying as a function of the heating time and temperature and the concentration of WPI. In the present study, the preheat treatment was applied before or after emulsion formation.

6.3 Materials and methods

Whey protein isolate (WPI), soybean oil and maltodextrin (MD) were used in this study. The details of these materials and the equipment used in the study have been described in Chapter 3 (section 3.1 and 3.2). Figure 6.1 a and b show the process flow diagrams for emulsion preparation, spray drying and analyses for emulsions described in section 6.3.1 and 6.3.2

6.3.1 Emulsion preparation - preheat treated WPI emulsions (PW)

WPI was dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solutions (0.5, 1.25, 3.0, 6.0 and 10%, w/w) were stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solutions were then adjusted to 7.0 using 1M HCl or 1M NaOH if required. These solutions were dispensed into separate 250 mL glass bottles (Schott AG, Mainz, Germany) and preheated to the desired temperature between 65 and 90ºC by introducing the bottles in a steam-jacketed kettle. A digital thermometer with thermocouple wire was used to record the temperature of the solution. While the solution was being heated, the bottles were being gently swirled for efficient heat distribution in the solution. As soon as the desired temperature was reached (3–4 min), the solutions were transferred to another water bath that was pre-set at the desired temperature (between 65 and 90ºC) and were held for 10 min. Following this, the solutions were cooled to room temperature by immediately placing the glass bottles in an ice bath (Fig. 6.1 a)

MD solution was prepared using the same stirring equipment with stirring until the powder was dissolved completely. All preheat treated WPI solutions were then mixed with soybean oil (20%, w/w) and maltodextrin solution (12%, w/w) and homogenized using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsions were further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Approximately, 500 mL of emulsion was prepared for each variation for spray drying purposes.

6.3.2 Emulsion preparation – heat treated WPI emulsions (HE)

WPI was dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solution was stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solution was then adjusted to 7.0 using 1M HCl or 1M NaOH if required. Similarly, MD solution was prepared using the same stirring equipment with stirring until the powder was dissolved completely. Soybean oil at 20.0% (w/w) was blended with the aqueous solutions of protein and MD using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Approximately, 500 mL of emulsion was prepared for each variation for spray drying purposes.

WPI emulsions at selected concentrations (0.5 and 3.0%, w/w) were dispensed into 500 mL glass bottles (Schott AG, Mainz, Germany) and heat treated to the desired temperature between 65 and 90ºC by introducing the glass bottles in a steam-jacketed kettle. A digital thermometer with thermocouple wire was used to record the temperature of the emulsions. While the emulsions were being heated, the bottles were gently swirled for efficient heat distribution within the emulsions. As soon as the desired temperature had been reached (3–4 min), the emulsions were transferred to another water bath that was pre-set at that desired temperature and were held for 10 min. Following this, the emulsions were cooled immediately in an ice bath to room temperature. Another set of emulsions that were made with the selected concentrations of WPI was heated at 90ºC for 1–30 min (Fig. 6.1b).

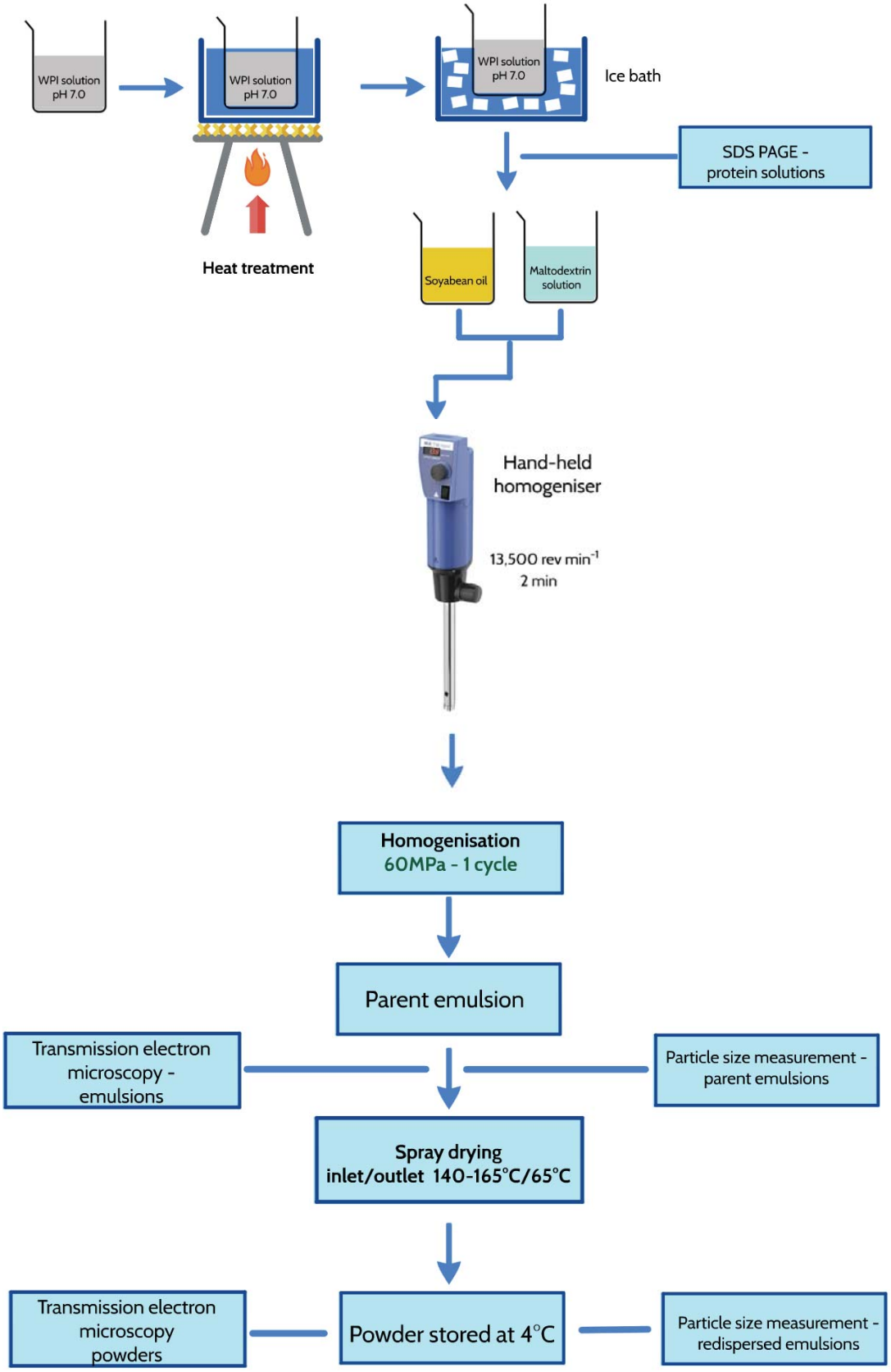


Figure 6.1a *Process flow diagram showing the emulsion preparation, spray drying and analyses for PW emulsions for this study.*

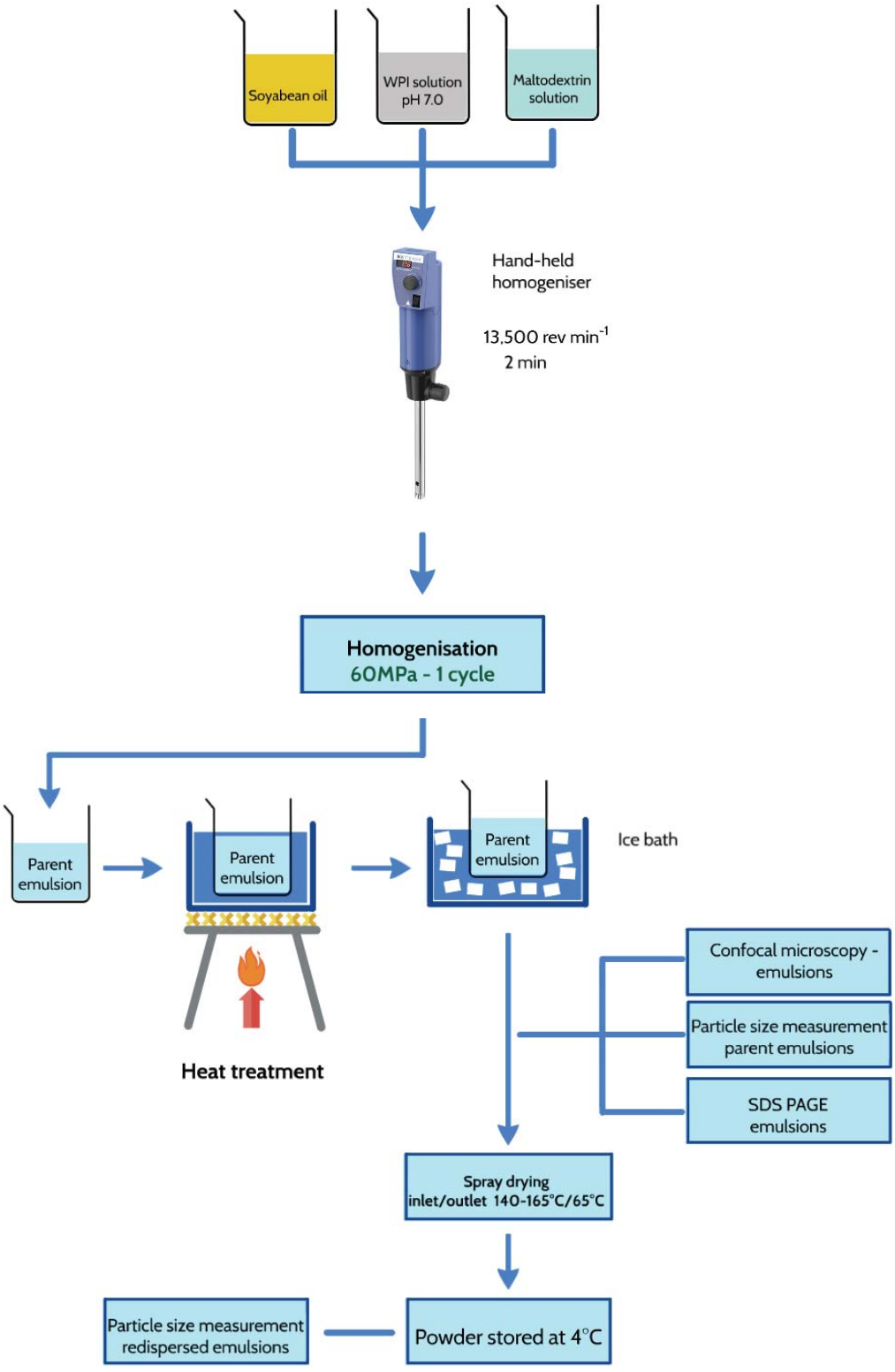


Figure 6.1b *Process flow diagram showing the emulsion preparation, spray drying and analyses for HE emulsions for this study.*

6.3.2 Spray drying

All emulsions were spray dried using a Buchi bench-top spray drier (B-290, Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current configuration and fitted with a two-fluid nozzle (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The inlet temperature varied between 140 and 165 °C and the outlet temperature was maintained at 65 °C. The resulting powders were collected in air-tight containers, allowed to cool to ambient temperature and then stored at 4 °C for up to 6 mths.

6.3.4 Determination of average droplet size

The diameters of the oil droplets for both the parent emulsions (fresh emulsions which have not been spray dried) and the redispersed emulsions (emulsions prepared by dissolving powder in RO water) were determined by the laser light scattering method as described by Jafari et al. (2007) and Ye (2011) using a Mastersizer E (Malvern Instruments, Malvern, Worcestershire, UK). Redispersed emulsions were prepared by reconstituting the dried powder in RO water to obtain the same total solids content as the parent emulsion. Powder was slowly added to RO water and the dispersions were stirred for at least 30 min before particle size measurements. In some cases, the emulsions were dispersed in 2.0% (w/w) SDS before the measurements. The comparison of the size distributions in RO water alone and with added SDS indicated flocculation/aggregation during emulsification/redispersion (Sanchez & Patino, 2005).

6.3.5 Confocal scanning laser microscopy

About 500 μL of emulsion (both parent and redispersed) sample was taken in an Eppendorf tube, 10 μL of Nile red (fluorescent dye, 0.1% w/v in acetone) was

added and the sample was gently shaken. A volume of 60 µL from this sample was placed on a concave microscope slide. A cover slip was carefully placed and the sample was observed under the microscope.

6.3.6 Transmission electron microscopy

A method similar to that described by McKenna et al. (1999) with some modifications was used for the powdered emulsion samples. Powdered emulsions were fixed with 25% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h at room temperature. After three washes in the same buffer, the samples were post-fixed with 1% (w/v) OsO_4 in the same buffer for 1 h at room temperature. The three buffer washes were repeated. The samples were then dehydrated using an acetone/water series (25, 50, 75, 95 and 100%) and were kept in each gradient for 10 min and in the 100% gradient for 2 h. The samples were first embedded with an acetone: resin (Procure 812) mixture (50%:50%) on a stirrer overnight, and then the acetone: resin was replaced with fresh 100% resin for another 8 h on the stirrer. The samples were finally mounted in 100% fresh resin at 60°C for 48 h. Sections 1 µm in thickness were cut from trimmed resin blocks using a glass knife and an Ultra microtome (Leica, Vienna, Austria). They were heat mounted on to glass slides, stained with 0.05% Toluidine Blue and viewed under a light microscope (Olympus BX51, Japan). Digital images of the sections were taken and areas of interest were chosen for examination using transmission electron microscopy. Ultra-thin sections (100 nm) were cut using a diamond knife and an Ultra microtome. They were collected on a copper grid. The sections were stained with saturated uranyl acetate in 50% ethanol for 4 min and then with lead citrate for another 4 min. They were examined using a Philips CM 10 transmission electron microscope (Philips, Eindhoven, Netherlands).

6.3.7 Polyacrylamide gel electrophoresis (PAGE)

The extent of aggregation of both the adsorbed protein and the non-adsorbed protein in the aqueous phase of preheated emulsions was determined using SDS polyacrylamide gel electrophoresis (SDS-PAGE), as described by Ye & Singh (2000). For non-reduced gels, a certain amount of emulsion was mixed with SDS buffer (0.5 M Tris, 2.0% SDS, pH 6.8), which was then centrifuged (13,000 *g* for 10 min, Sorvall RC-5C, DuPont Co., Wilmington, DE, USA) to separate the cream layer. For reduced gels, SDS buffer, containing 0.05% 2-mercaptoethanol, was added to emulsions, which were heated to 95ºC for 5 min and centrifuged as above. The samples for both types of gel were cooled to room temperature and a portion (20 μL) of the subnatant was applied to SDS gels (Criterion Precast Gels, 10–20% Tris-Tricine, Bio-Rad Laboratories, Hercules, CA, USA) at 150 V using a power supply unit (PowerPac Basic, Bio-Rad Laboratories, Richmond, CA, USA). Preheated WPI solutions were also mixed with the two SDS buffers in the same way as the heated emulsions and a portion (20 μL) of the dispersion were applied to SDS gels as described above. All gels were then stained with Coomassie Blue R-250 (0.05%, w/v, in 25%, v/v, isopropanol, 10%, v/v, acetic acid) for 30 min and then destained using a destaining solution (10%, v/v, isopropanol, 10%, v/v, acetic acid). These gels were scanned using a gel imaging system (Gel Doc System, Bio-Rad Laboratories, Richmond, CA, USA).

6.3.8 Densitometry

The intensities corresponding to different temperature and times were measured using the volume tool in Image Lab software (Bio-Rad Laboratories, Richmond, CA, USA). The intensities were corrected against background intensities using local background subtraction. The percentage composition of each band of β-

lactoglobulin and α-lactalbumin were determined by scanning the areas and comparing against the intensities of the unheated bands which were considered as 100%.

6.3.9 Data analysis

Each data point presents average value of two determinations on two separate emulsions (measured in duplicates). Error bars in graphs represent standard deviation of average values of two determinations on separate emulsions. Some graphs were drawn using Sigmaplot 13 graphing and data analysis software (Systat Software Inc., San Jose, CA, USA) and the remaining graphs were drawn using Microsoft Excel 2011(Microsoft Corporation, WA, USA).

6.4 Results

6.4.1 Effect of heating temperature and time on emulsion properties

6.4.1.1 Preheating of WPI solutions (PW)

Figure 6.2 shows the average droplet diameters (d_{43}) of PW emulsions (0.25–5.0%, w/w, WPI; 20.0%, w/w, soybean oil; 12%, w/w, maltodextrin; pH 7.0) before and after spray drying. These emulsions were prepared using either unheated or preheat-treated WPI solutions with concentrations ranging between 0.5% and 10.0% (w/w). For the emulsions made using unheated WPI solutions, the d_{43} values were comparable with the findings in Chapter 4.

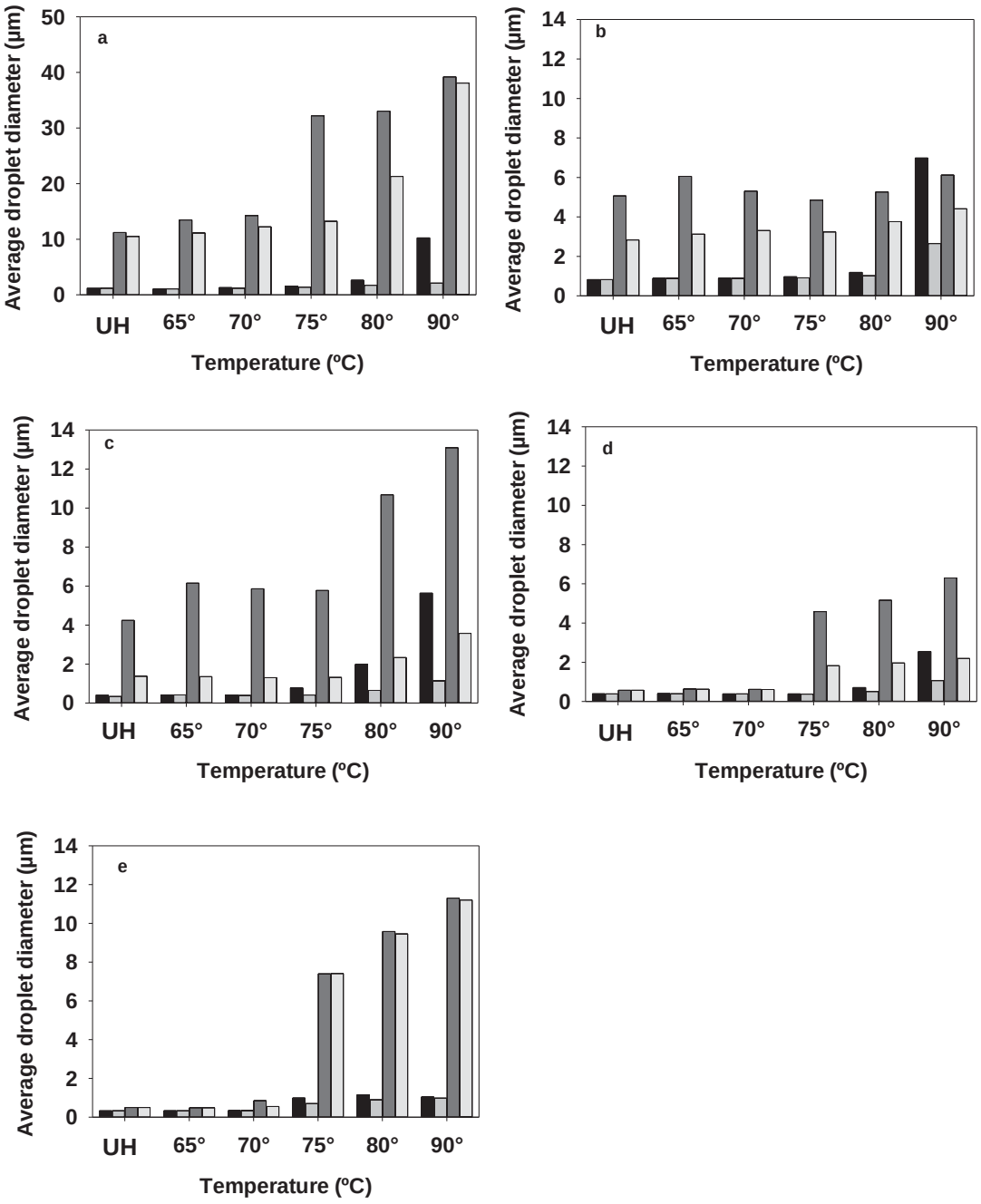


Figure 6.2 *Average droplet diameters (d_{43}) values of PW emulsions (20%, w/w, soybean oil; 12%, w/w, maltodextrin) containing 0.25–5.0% (w/w) WPI as a function of the preheat temperature (65–90ºC) for 10 min before spray drying (■) and after redispersion of the powder in water (■). Also, d_{43} of emulsions upon dilution (1:1) with 2.0% (w/w) SDS solution before spray drying (□) and after redispersion (□). The WPI concentrations in the emulsions were: a, 0.25%; b, 0.5%; c, 1.25%; d, 3.0%; e, 5.0%. UH denotes unheated emulsions.*

Preheat treatment of the protein solutions at ≤ 70ºC resulted in no change in the d_{43} values in all parent emulsions. Preheat treatment at 75 and 80ºC resulted in a slight increase in the d_{43} values for some emulsions but preheat treatment at 90ºC resulted in a significant increase in the d_{43} values for all emulsions. The only exception was the emulsion containing 5.0% (w/w) WPI, for which there was no noticeable change in d_{43} value compared with the emulsion containing 5.0% (w/w) WPI and preheat treated at 80ºC.

When the emulsions were diluted (1:1) with 2.0% (w/w) SDS solution (which breaks hydrophobic interactions and displaces protein molecules from the oil–water interface), there was no noticeable change in the d_{43} values for the emulsions prepared using unheated protein solutions. Similar results were also obtained for all emulsions prepared using preheated WPI solutions, except for those containing 0.25–3% (w/w) WPI preheat treated at 90ºC. This indicated that the increase in d_{43} upon heating at > 80°C was due to the aggregation/flocculation of oil droplets (Fig. 6.2).

Upon spray drying and redispersion of these PW emulsions in RO water, there was a marked increase in the d_{43} values, compared with their corresponding parent emulsions containing ≤ 1.25% (w/w) WPI. Some aggregation/flocculation had occurred in these emulsions, as the droplet diameter shifted towards smaller values when they were diluted (1:1) with 2.0% (w/w) SDS solution. However, most of the shift towards the larger size range was due to droplet re-coalescence post spray drying. For the untreated emulsions containing ≥ 3.0% (w/w) WPI, the d_{43} values before and after spray drying were comparable, in agreement with the findings of Chapter 4. Preheat treatment at ≤ 70ºC did not result in any noticeable change in the

d_{43} values of the redispersed emulsions compared with the corresponding unheated redispersed emulsions.

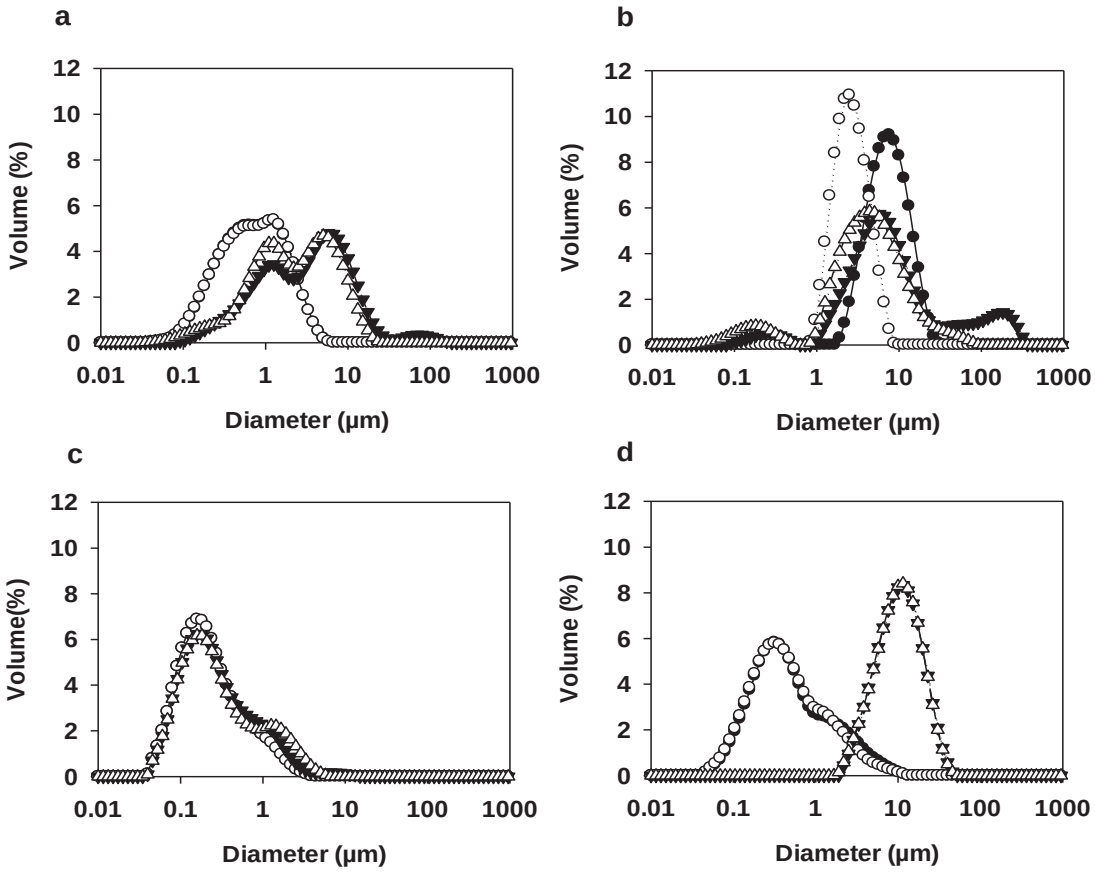


Figure 6.3 *Droplet size distributions of emulsions (20%, w/w, soybean oil; 12%, w/w, maltodextrin) containing 0.5% (w/w) (a, b) and 5.0% (w/w) (c, d) WPI. These emulsions were prepared using either unheated WPI solutions (a, c) or preheated WPI solutions (b, d) at a preheating temperature of 90ºC for 10 min. Parent emulsions (●), parent emulsions diluted (1:1) with 2.0% (w/w) SDS solution (○), redispersed emulsions (▼) and redispersed emulsions diluted (1:1) with 2.0% (w/w) SDS solution (Δ).*

However, preheat treatment at above 70°C resulted in a noticeable increase in the d_{43} values of all redispersed emulsions compared with the corresponding untreated redispersed emulsions. Above 70ºC, as the WPI concentration in the preheat-treated redispersed emulsions was increased, the d_{43} values gradually decreased; the exception was the redispersed emulsions containing 5.0% (w/w) WPI, for which the d_{43} values increased markedly. There was no change in the d_{43} values in these emulsions when they were diluted with 2.0% (w/w) SDS solution, suggesting that the most likely cause of the increase in droplet diameter post spray drying was oil droplet re-coalescence (Fig. 6.2). Interestingly, there was very little change in the d_{43} values because of preheat treatment in the redispersed emulsions containing 0.5% (w/w) WPI.

Fig. 6.3 shows the droplet size distributions of parent and redispersed emulsions made using either unheated WPI solutions or WPI solutions preheated at 90ºC for 10 min at selected concentrations of WPI in the emulsion (0.5 and 5.0%, w/w). The size distributions of the emulsions prepared using unheated protein solutions before and after spray drying became narrower and shifted towards a smaller size range with an increase in the WPI concentration (Figs. 6.3 a, c). The size distributions of the emulsions containing 5.0% (w/w) WPI were bimodal and almost overlapped before and after spray drying (Fig. 6.3 c), which was consistent with the findings in Chapter 4. At 0.5% (w/w) WPI, compared with the unheated parent emulsions, the parent emulsions prepared using WPI solutions heated at 90ºC for 10 min had narrower, monomodal size distributions that showed a marked shift towards a larger size range. This shift was further amplified when these emulsions were spray dried and redispersed; the distribution became multimodal and noticeably broader. At 5.0% (w/w) WPI, although the size distributions became narrower and monomodal

upon spray drying and redispersion, there was a large shift towards the larger size range (Fig. 6.3 d).

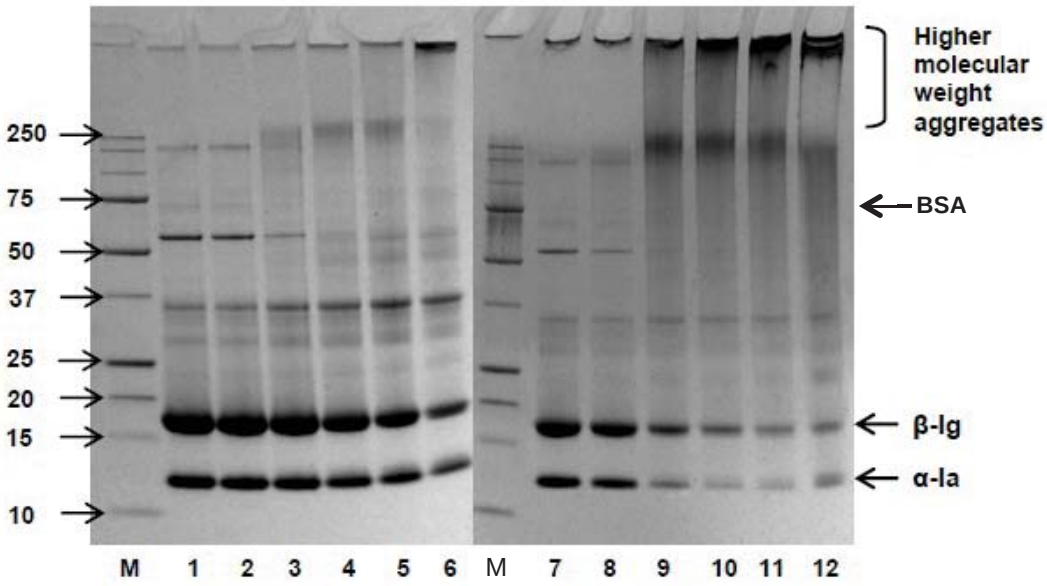


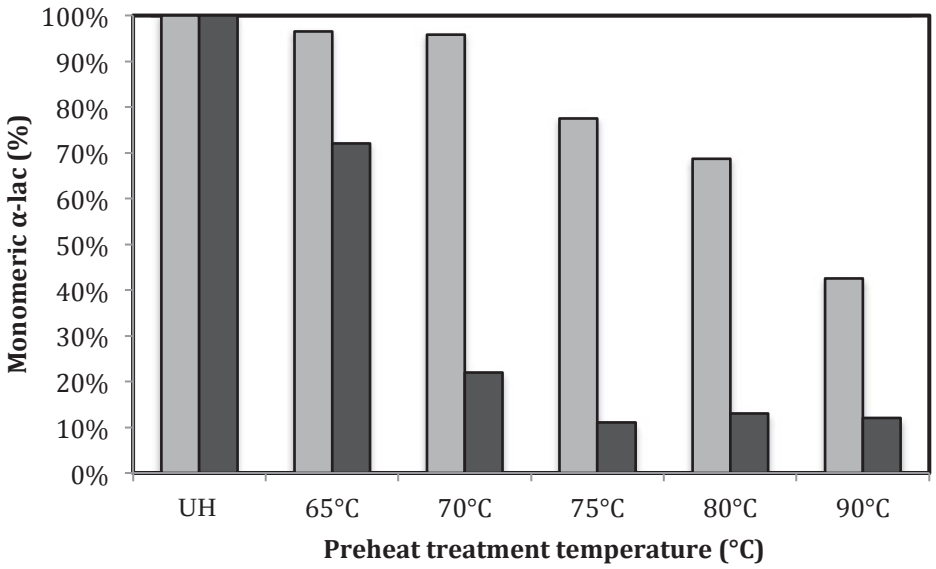
Figure 6.4. *SDS-PAGE patterns (under non-reducing conditions) of WPI solutions at concentrations of 1.25% (w/w) (lanes 1–6) and 10.0% (w/w) (lanes 7–12) that were heated for 10 min at different temperatures: lanes 1 and 7, unheated; lanes 2 and 8, 65ºC; lanes 3 and 9, 70ºC; lanes 4 and 10, 75ºC; lanes 5 and 11, 80ºC; lanes 6 and 12, 90ºC. M denotes the lane with the molecular weight marker. BSA denotes bovine serum albumin.*

SDS-PAGE of unheated and preheat-treated WPI solutions with concentrations of 1.25% and 10.0% that were used for the preparation of emulsions containing 0.5 and 5.0% (w/w) WPI respectively was performed to examine the extent of the loss of native α-la and β-lg because of denaturation and aggregation. The SDS-PAGE

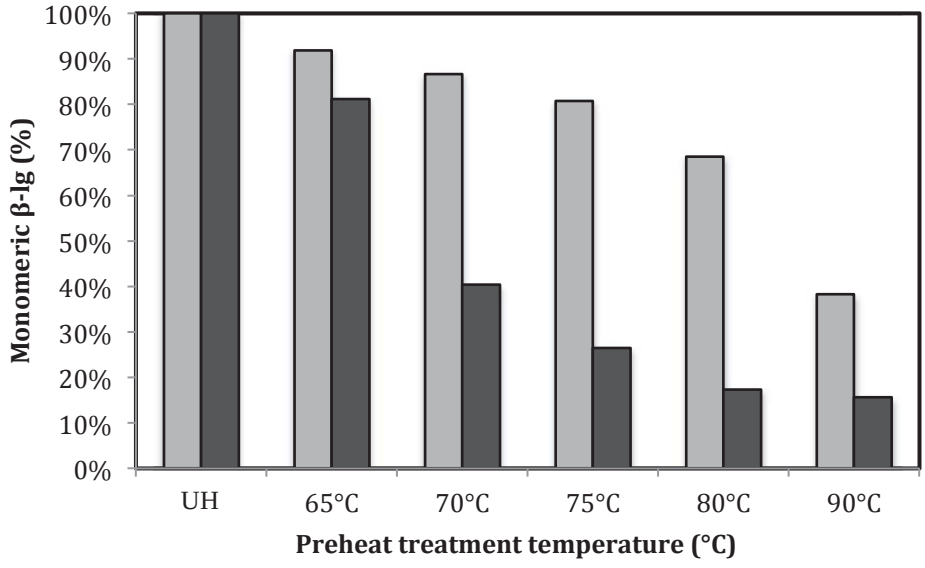
(under non-reducing conditions) patterns of the unheated protein solutions (lanes 1 and 7; Fig. 6.4) showed three major protein bands for monomeric α-la, β-lg and BSA, corresponding to molecular weights of approximately 14, 18 and 67 kDa respectively (Damodaran & Agyare, 2013; Havea et al., 1998). Compared with the unheated WPI solutions, at both WPI concentrations, the protein band corresponding to BSA completely disappeared (lanes 3 and 9; Fig. 6.4) with an increase in the preheat treatment above 70ºC. This was accompanied by a decrease in the intensity of the monomeric α-la and β-lg bands and the appearance of protein bands representing polymeric species corresponding to molecular weights of > ~ 50 kDa. Higher molecular weight species that could not enter the resolving portion of the gel were apparent at both WPI concentrations above a preheat temperature of 70ºC; however, these bands were much more intense in the 10.0% (w/w) WPI solution, even at 70ºC (Fig. 6.4).

The band intensities of monomeric α-la and β-lg were noticeably lower in both WPI solutions (1.25 and 10.0%, w/w) at a preheating temperature of 90ºC than in the unheated solution (Fig. 6.4). The total monomeric α-la that remained at the end of the preheat treatment at 75ºC was ~ 11% in the 10.0% (w/w) WPI solutions and was ~ 77% in the 1.25% (w/w) WPI solutions (Fig. 6.5 a). For the 1.25% (w/w) WPI solutions, ~ 80% monomeric β-lg remained at a preheat temperature of 75ºC, but this decreased to ~ 38% with an increase in the preheat temperature to 90ºC. In contrast, for the 10% (w/w) WPI solutions, the decrease in monomeric β-lg was much more rapid, with only ~ 27% remaining at a preheat temperature of 75ºC and ~ 16% remaining at a preheat temperature of 90ºC (Fig. 6.5 b). This, along with a higher proportion of larger particles that were unable to enter the stacking portion of the gels, was indicative of a marked increase in the rate/extent of aggregation

with an increase in the concentration of whey protein in the solutions (Figs. 6.4 and 6.5).



a)



b)

Figure 6.5 *Percentages of residual monomeric (a) α-la and (b) β-lg remaining in 1.25% (w/w) (□) and 10.0% (w/w) (■) WPI solutions that were preheated for 10 min at various preheating temperatures. UH denotes unheated solutions.*

6.4.1.2 Heating of emulsions (HE)

During the manufacture of powdered emulsions, heat treatment is often applied to the emulsions after homogenization but prior to spray drying. Therefore, to examine the impact of heat treatment on WPI-stabilized emulsions, another set of emulsions was prepared. The WPI solutions were not subjected to any preheat treatment. Instead, heat treatment, similar to that applied to WPI solutions, was applied to the emulsions (HE) after the homogenization step (Fig 6.1 b). These emulsions were prepared using only two WPI concentrations, i.e. 0.5 and 3.0% (w/w), which were selected based on the results of the previous experiments and were representative of poor and good stability during spray drying respectively (Chapter 4). Heat treatment at between 65 and 90ºC did not affect the d_{43} values of the parent emulsions containing 0.5% and 3.0% (w/w) WPI. As expected, there was no change in the droplet diameter in both parent emulsions upon dilution with 2.0% (w/w) SDS solution, suggesting no aggregation (inter-droplet) amongst the oil droplets (Fig. 6.6).

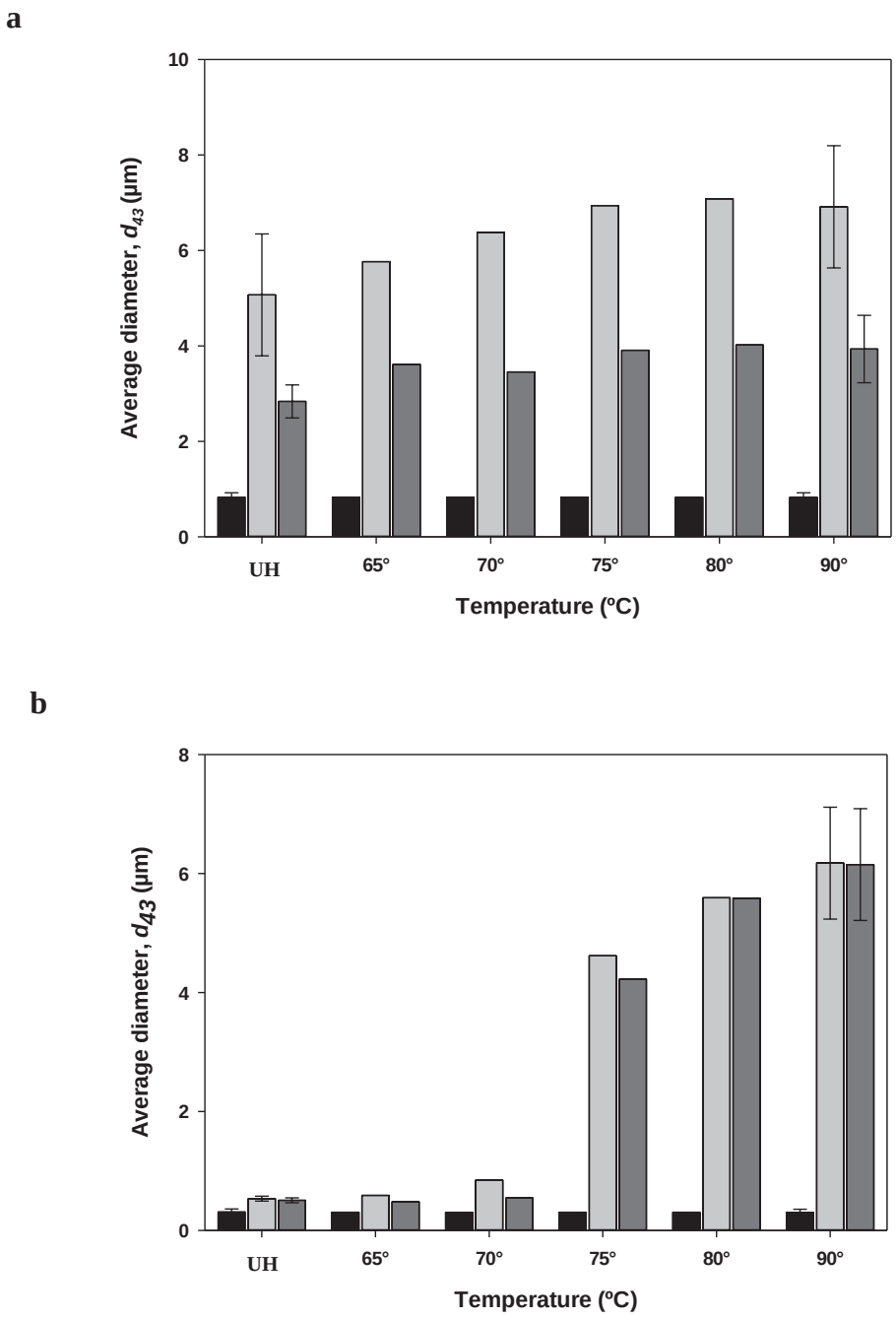


Figure 6.6 *Average droplet diameters (d_{43}) of HE emulsions (20%, w/w, soybean oil; 12.0%, w/w, maltodextrin) containing (a) 0.5% (w/w) and (b) 3.0% (w/w) WPI as a function of heating temperature (65–90ºC) for 10 min before spray drying (■) and after redispersion of the powder in water (□). Average droplet diameter (d_{43}) values of redispersed emulsions upon dilution (1:1) with 2.0% (w/w) SDS solution (▣). UH denotes unheated emulsions.*

Upon spray drying and redispersion, the unheated HE emulsions containing 0.5% (w/w) WPI showed a marked increase in d_{43} value compared with the corresponding parent emulsions. However, no noticeable increase in d_{43} value was seen with an increase in the temperature from 65 to 90ºC (Fig. 6.6a). When the redispersed emulsions were diluted with 2.0% (w/w) SDS solution, although there was a considerable decrease in the d_{43} values (about 50%), the values were still much higher than those of the parent liquid emulsions. This indicated that the marked shift in the d_{43} values was due mainly to droplet re-coalescence. Confocal micrographs of redispersed emulsions containing 0.5% (w/w) WPI and heated at 90ºC before spray drying showed aggregated droplets, which appeared to dissociate when the emulsion was diluted with SDS solution (Fig. 6.7).

The redispersed emulsions containing 3.0% (w/w) WPI showed no noticeable change in their d_{43} values when heated up to 70ºC and had comparable droplet diameter values before and after spray drying, which was consistent with the previous findings in Chapter 4. There was a gradual increase in the d_{43} above 70ºC and the average values at 90ºC were larger for the preheat-treated redispersed HE emulsions containing 3.0% (w/w) WPI than for the preheat-treated redispersed HE emulsions containing 0.5% (w/w) WPI. There was no change in the d_{43} values of the redispersed HE emulsions containing 3.0% (w/w) WPI upon dilution with SDS solution, which suggested that the shift towards larger d_{43} values was mainly due to oil droplet re-coalescence (Fig. 6.6 b).

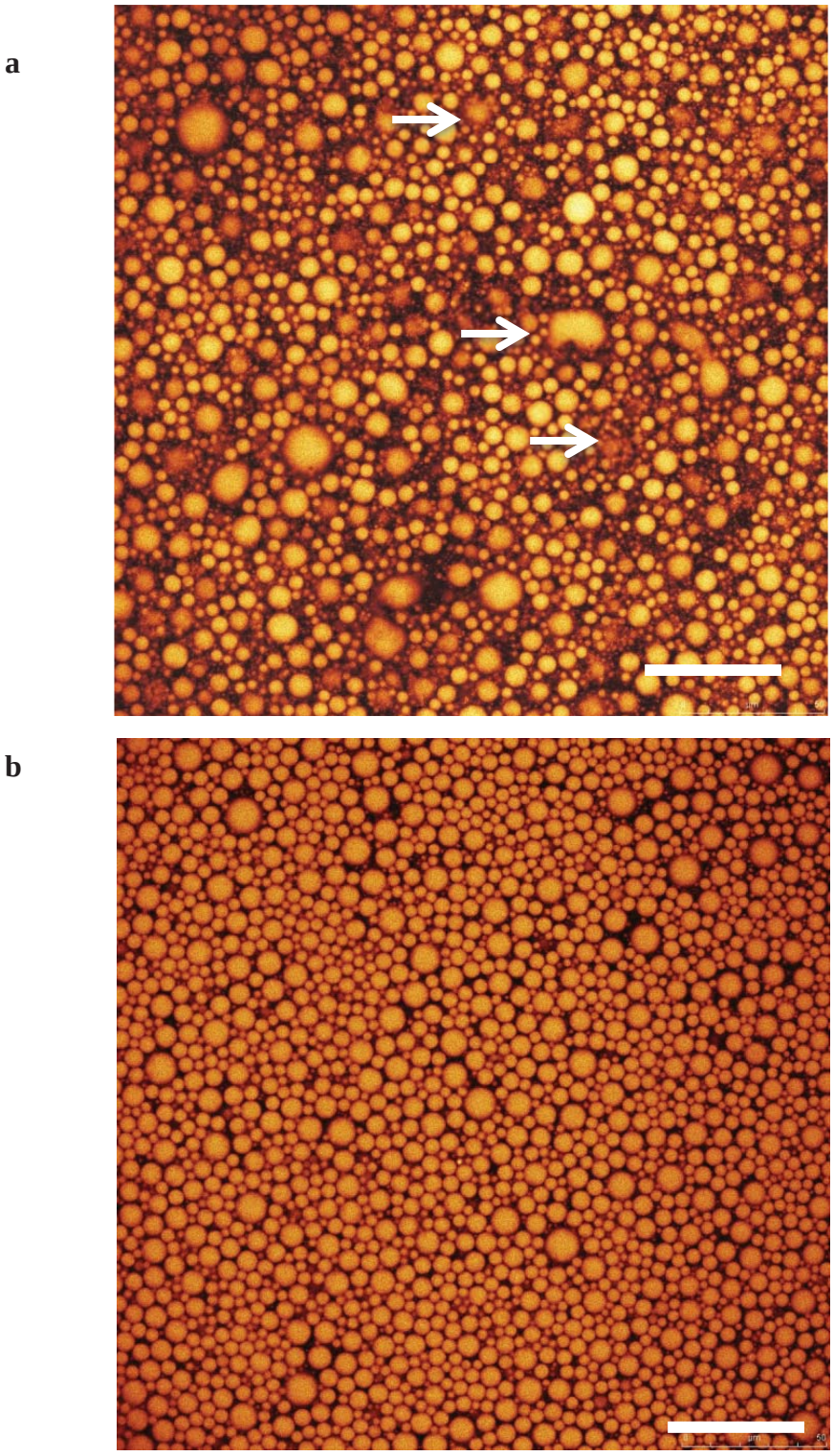


Figure 6.7 *Confocal micrographs of HE emulsions (0.5%, w/w, WPI; 20%, w/w, soybean oil; 12%, w/w, maltodextrin) heated at 90ºC for 10 min after spray drying and redispersion (a) in water and (b) diluted (1:1) in 2.0% (w/w) SDS. Arrows show some of the aggregates present in the micrograph (a). Scale (white bar) is 50μm.*

When the HE emulsions containing 0.5% (w/w) WPI were compared with the PW emulsions (Section 6.4.1.1) prepared using 0.5% (w/w) WPI solutions (0.25%, w/w, WPI in the emulsion, so that the concentrations of protein in the emulsions and solutions were the same during heating), the shift in the particle size towards the larger size range was much greater in the PW emulsions, even though the concentration of WPI during heat treatment of the solutions and the emulsions was similar. However, the resulting d_{43} values of the redispersed HE emulsions containing 0.5% (w/w) WPI were comparable with those of the redispersed PW emulsions containing 0.5% (w/w) WPI (1.25%, w/w, WPI in the solution during heating). Similarly, the d_{43} values of the redispersed HE emulsions containing 3.0% (w/w) WPI were compared with those of the redispersed PW emulsions prepared using preheat-treated 3.0% (w/w) WPI solution (1.25%, w/w, WPI in the emulsion) at corresponding heat treatment temperatures above 70ºC (Figs. 6.2 and 6.6). The redispersed HE emulsions containing 3.0% (/w) WPI had slightly higher average droplet diameters than the redispersed PW emulsions prepared using 3.0% (w/w) preheat-treated WPI solutions (1.25%, w/w, WPI in the emulsion).

SDS-PAGE was also performed on the parent HE emulsions to examine the extent of aggregation of the adsorbed and unadsorbed proteins as a function of preheat treatment temperature. There was a decrease in the intensity of the monomeric α-la and β-lg bands at both concentrations of WPI in the emulsion. However, the decrease in the monomeric proteins and the appearance of polymeric species (~ 250 kDa) appeared to be much faster at 3.0% (w/w) WPI than at 0.5% (w/w) WPI in the HE emulsions at the same heating temperature (Fig. 6.8). Residual monomeric β-lg appeared to be as low as ~ 28% at 75°C, which seemed to further decrease to ~ 17% at 90ºC. At a preheat treatment temperature of 90ºC, the percentages of

residual monomeric α-la and β-lg in the HE emulsions containing 0.5% (w/w) WPI were ~ 60% and ~ 40% respectively (Fig. 6.9).

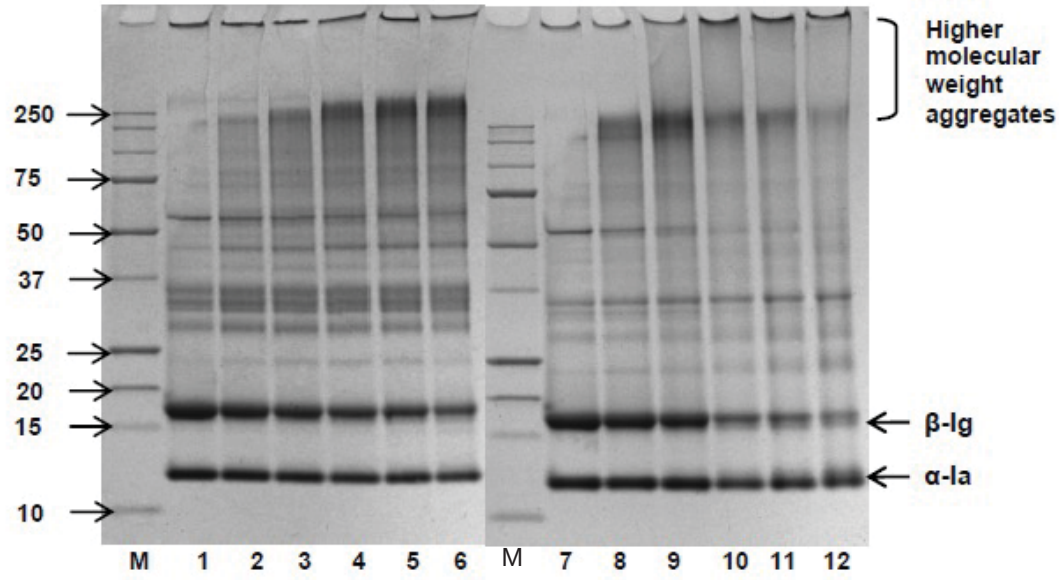
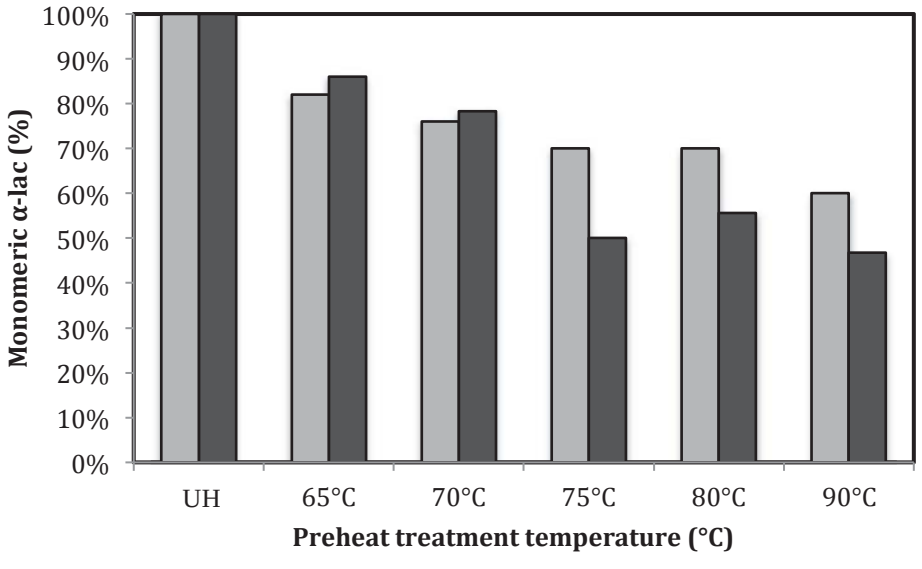
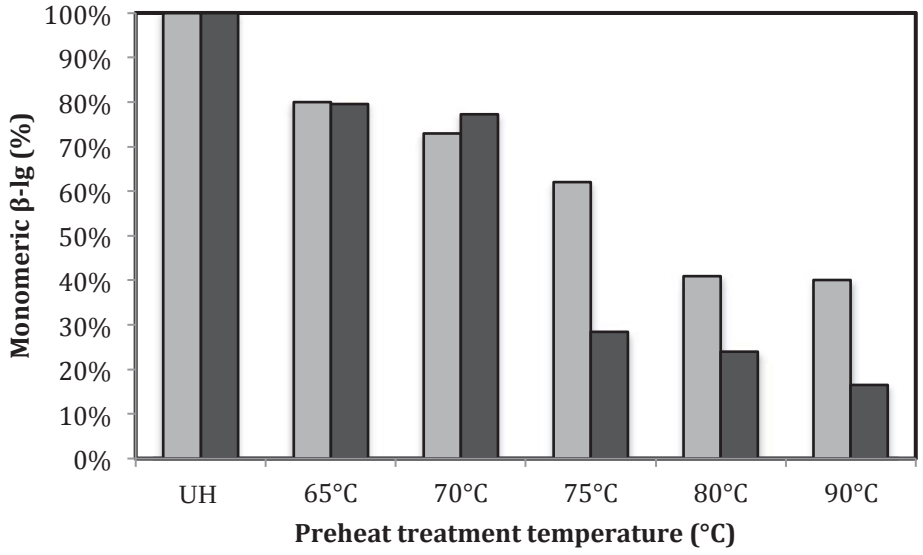


Figure 6.8 *SDS-PAGE patterns (under non-reducing conditions) of emulsions stabilized with 0.5% (w/w) (lanes 1–6) and 3.0% (w/w) (lanes 7–12) WPI that were heated for 10 min at different temperatures: lanes 1 and 7, unheated; lanes 2 and 8, 65ºC; lanes 3 and 9, 70ºC; lanes 4 and 10, 75ºC; lanes 5 and 11, 80ºC; lanes 6 and 12, 90ºC. M denotes the lane with the molecular weight marker.*



a)



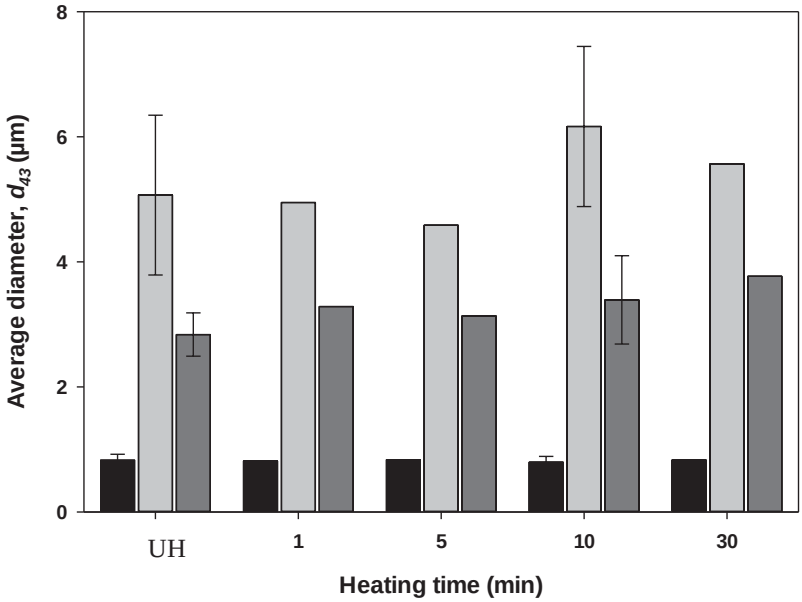
b)

Figure 6.9 *Percentages of residual monomeric (a) α-la and (b) β-lg remaining in whey-protein-stabilized emulsions at WPI concentrations of 0.5% (w/w) (□) and 3.0% (w/w) (■) that were preheat treated for 10 min at various preheating temperatures. UH denotes unheated emulsions.*

6.4.2 Effect of heating time

The effect of heating time on the average droplet diameter in WPI-stabilized emulsions was also studied. As the lowest amount of residual monomeric protein was observed for HE emulsions containing 0.5 and 3.0% (w/w) WPI at 90ºC, this temperature was chosen to study the effect of temperature as a function of time (1–30 min). With an increase in heating time from 1 to 30 min, there was no noticeable change in d_{43} values of the parent HE emulsions at both 0.5 and 3.0% (w/w) WPI. Upon spray drying and redispersion, compared with the unheated system, the HE emulsions containing 0.5% (w/w) WPI again showed no noticeable change in the d_{43} values after 1 min of heating and no further change with an increase in the heating time (Fig. 6.10 a). For the HE emulsions containing 3.0% (w/w) WPI, the increase in the d_{43} values following spray drying and redispersion was marked after heating for 1 min but the d_{43} values did not change further with an increase in the heating time from 1 to 30 min (Fig. 6.10 b).

a



b

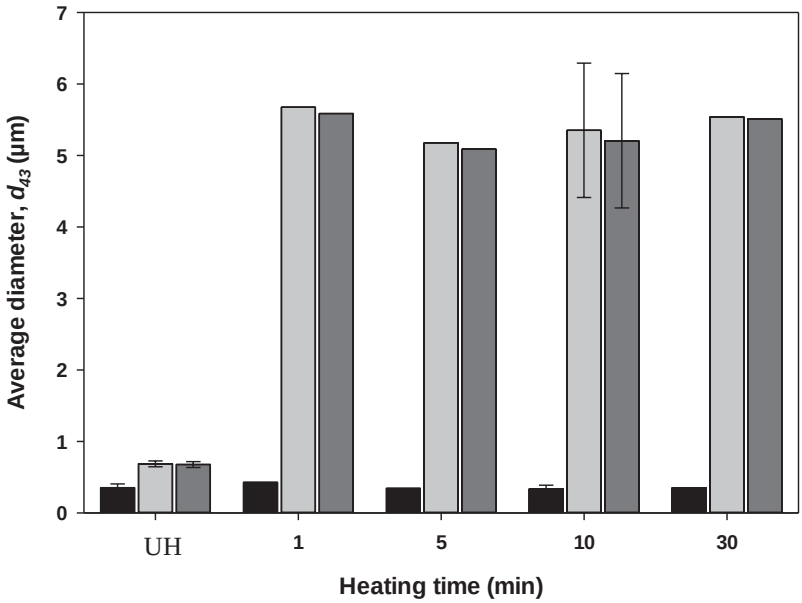
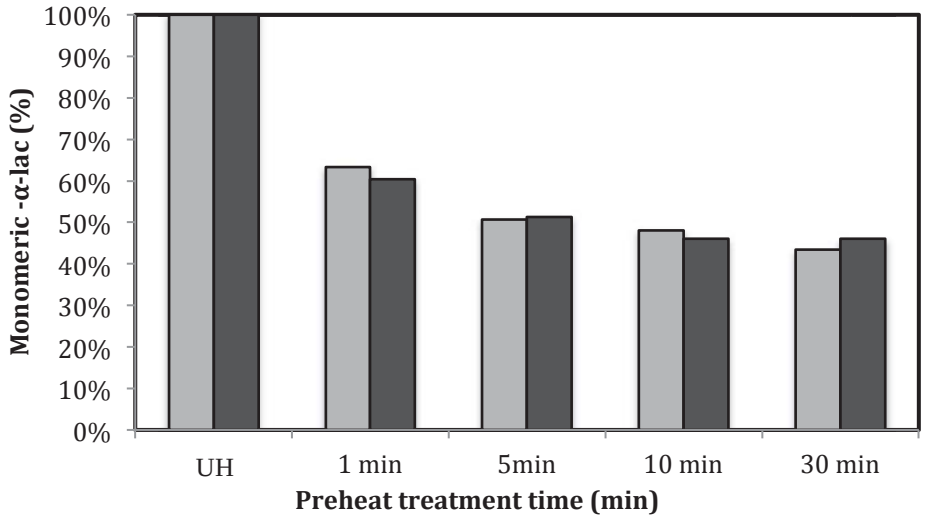


Figure 6.10 *Average droplet diameters (d_{43}) values of emulsions (20%, w/w, soybean oil; 12%, w/w, maltodextrin) containing (a) 0.5% (w/w) and (b) 3.0% (w/w) WPI as a function of heating time (1–30 min) at 90ºC before spray drying (■) and after redispersion of the powder in water (□). Average droplet diameters (d_{43}) values of redispersed emulsions after dilution (1:1) with 2.0% (w/w) SDS solution (■). The emulsions were heat treated after homogenization. UH denotes unheated emulsions.*

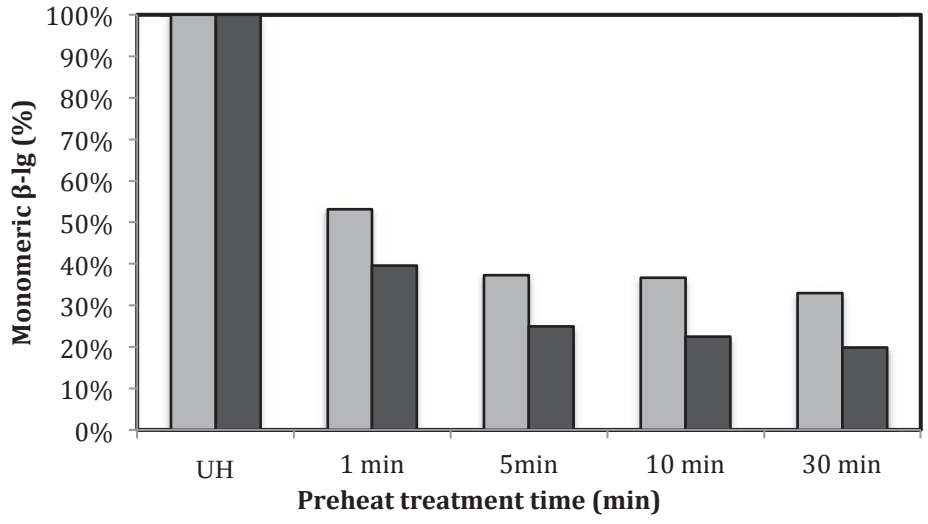
The SDS-PAGE patterns of the HE emulsions containing 0.5% (w/w) WPI showed that ~ 60% of the monomeric α-la and ~ 50% of the monomeric β-lg remained after 1 min of heating at 90ºC and that about ~ 50% and ~ 40% respectively remained after 5 min (Fig. 6.11). There was no marked change in the residual monomeric species with further heating to 30 min. For the HE emulsions containing 3.0% (w/w) WPI, the residual monomeric species followed a similar trend, with no marked decrease in the residual monomeric α-la and β-lg upon further heating past 5 min (Fig. 6.11).

6.4.3 Transmission electron microscopy

Individual emulsion droplets, with droplet diameters ranging from 50 to 1000 nm, were clearly visible in micrographs of the untreated emulsions (Figs. 6.12a and 6.12b). The surfaces of the droplets contained some dark (electron-dense) particles, which were probably small protein aggregates. Even though the average droplet diameter was in a similar range for the heat-treated emulsions (Figs. 6.12c and 6.12d), the number and the size of the darker electron-dense particles were noticeably greater. Furthermore, the droplets seemed to be closely packed and bridging flocculation could be observed.



a



b

Figure 6.11 *Percentages of residual monomeric (a) α-la and (b) β-lg remaining in whey-protein-stabilized emulsions at WPI concentrations of 0.5% (w/w) (□) and 3.0% (w/w) (■) that were preheat treated for 1–30 min at a preheating temperature of 90ºC. UH denotes unheated emulsions.*

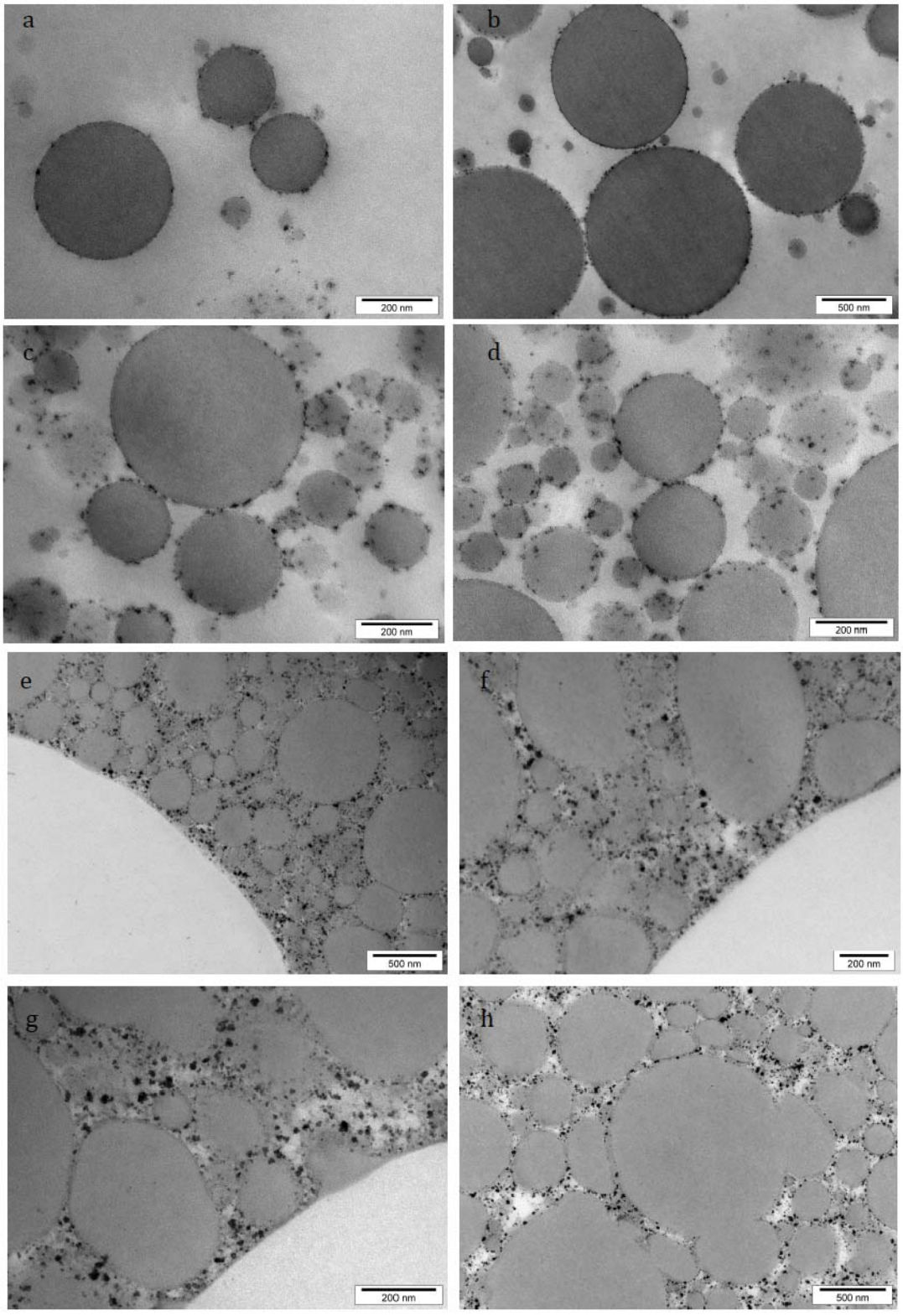


Figure 6.12 *Transmission electron micrographs of (a–d) emulsions and (e–h) powders containing 3.0% (w/w) WPI and either untreated (a, b, e, f) or preheat treated at 90ºC for 10 min (c, d, g, h). Scale as shown in the micrographs. White areas marked with arrows in some micrographs (e, f, g) represent vacuoles or air cavities at the centre of a powder particle.*

In the micrographs of the powders (without dispersion in water), the effects of heating were much less distinguishable. The sample processing for microscopy resulted in partial dissolution of the surface of the powder particles. However, sections of the powder particles that were close to the central air cavity/vacuole were intact and are shown in Figs. 6.12 e-h. Although dark electron-dense particles were also visible in the micrographs of the powders, there were no obvious differences in intensity in the heat-treated powders compared with the untreated powders. In the heat-treated powders, oil droplets that had entered the air–water interface (air cavity) but possibly had not yet spread completely over the inside surface were observed (Fig. 6.12 g). Interestingly, there were also some semi-coalesced oil droplets, with what seemed like "neck" formations, in the matrix of particles for powders made from emulsions that were heated at 90ºC for 10 min (Fig. 6.12h). These partially coalesced droplets could probably coalesce into larger droplets upon redispersion.

6.5 Discussion

In this study, parent emulsions that were prepared using preheat-treated WPI solutions (65–90ºC for 10 min) showed a shift in their average droplet diameters towards the larger size range, especially at the highest heat treatment temperature of 90ºC (Figs. 6.2 and 6.3). This shift in d_{43} values was probably due to the depletion (in part) of native monomeric whey proteins and the formation of whey protein aggregates as a direct result of the heat treatment applied to these protein solutions (Figs. 6.4 and 6.5). The depletion rates for the two major whey proteins, β-lg and α-la, were higher at higher heat treatment temperatures (90ºC) at a given concentration of protein in solution (Figs. 6.4 and 6.5), which was in agreement

with the published literature (Dannenberg & Kessler, 1988; de la Fuente, Singh, & Hemar, 2002; Mehalebi, Nicolai, & Durand, 2008). The shift in the d_{43} values of the parent emulsions corresponded well with the depletion of monomeric β-lg and α-la, as the shift towards larger values was greater at higher preheat treatment temperatures (Figs. 6.2 and 6.3). Although whey protein aggregates present in the protein solutions could adsorb at the surface of the oil droplet during homogenization, their ability to efficiently cover the interface would be lower than that of the unheated monomeric proteins, especially at lower protein concentrations in solution (Euston & Hirst, 1999). Similar findings have been reported when solutions containing whey protein products (WPC, WPI) preheated above the denaturation temperature of β-lg (Millqvist-Fureby, Elofsson, & Bergenstahl, 2001; Dybowska, 2008).

As the concentration of protein in the preheat-treated WPI solutions was increased from 0.5 to 10.0 % (w/w), the average droplet diameter of the resulting parent emulsions showed a shift towards the smaller size range at corresponding preheat treatment temperatures (Figs. 6.2 and 6.3). This was possibly due to the increase in the amount of residual monomeric α-la and β-lg with the increase in total protein concentration in the preheat-treated solutions, which led to an improvement in emulsification efficiency (Figs. 6.4 and 6.5). From the PAGE results shown in Figs. 6.4 and 6.5, after heat treatment at 90ºC for 10 min, the percentages of monomeric α-la and β-lg remaining in solution were ~ 12% and ~ 16% respectively at 10% (w/w) WPI, compared with ~ 40% and ~ 38% respectively at 1.25% (w/w) WPI. The combined residual monomeric α-la and β-lg contents after the application of heat treatment were estimated to be ~ 11 and ~ 3.5 g L^{-1} for 10.0 and 1.25% (w/w) protein solutions respectively. There is an abundance of literature showing the

dependence of whey protein depletion on the temperature/time protocol and the concentration of protein in solution (Tolkach & Kulozik, 2005; Mahmoudi, Mehalebi, Nicolai, Durand & Riaublanc, 2007; Mounsey & O'Kennedy, 2007; Nicolai et al., 2011). Therefore, with an increase in the concentration of total protein in the solutions, the concentration of residual monomeric protein after heat treatment also increased, resulting in increased emulsification efficiency and a decrease in the d_{43} values.

Upon spray drying and redispersion of these emulsions, a shift in the size distribution towards the larger size range was observed for emulsions prepared using protein solutions that were preheated above 70ºC. This shift can be explained with the hypothesis proposed previously (Chapters 4 and 5). It was suggested that the concentration of unadsorbed monomeric protein in the aqueous phase must be above a critical value (~ 1.0%, w/w) so that the unadsorbed protein molecules can migrate from the bulk phase to the air–water interface during spray drying because of their high surface activity. Below this critical concentration of unadsorbed protein (~ 1.0%, w/w) in the bulk phase, protein molecules present at the oil–water interface could potentially desorb and migrate to the air–water interface, leaving "gaps" in the oil droplet interface, eventually resulting in droplet re-coalescence during drying.

At a given protein concentration, emulsions prepared using preheat-treated protein solutions would probably have lower amounts of unadsorbed monomeric protein in the bulk phase than emulsions prepared using unheated solutions because of heat-induced denaturation and aggregation. This could potentially result in the migration of some of the whey proteins from the oil droplet interface to the air–water

interface, leading to re-coalescence during drying and an increase in the d_{43} values of the redispersed emulsions (Figs. 6.2 and 6.3). That is, as seen in the preheat-treated parent emulsions, with an increase in the protein concentration in the emulsions from 0.25% to 3.0% (w/w), the d_{43} values of the redispersed emulsions showed a shift towards the smaller size range at the corresponding preheat treatment temperature (Figs. 6.2 and 6.3). This is also likely to be related to an increase in the concentration of unadsorbed monomeric protein in the bulk phase of the emulsions with an increase in the concentration of total protein.

In contrast, for emulsions prepared using preheat-treated 10% (w/w) protein solutions, the average droplet diameter value of the redispersed emulsions showed a reverse trend, i.e. the d_{43} values shifted towards the larger size range in emulsions that were preheat treated above 70°C compared with the redispersed emulsions with lower protein concentrations at the corresponding preheat treatment temperatures (Figs. 6.2 and 6.3). The SDS-PAGE patterns of the WPI solutions that were used to prepare the emulsions containing 5.0% (w/w) WPI showed that the extent of aggregation was much more severe and progressed at a much faster rate at temperatures > 70°C. Also, a large proportion of the proteins were aggregated and were unable to enter the stacking portion of the gel because of their large size. The estimated concentration of monomeric α-la and β-lg in 10.0% (w/w) protein solutions after preheat treatment at 90°C for 10 min was ~ 11 g L^{-1}. In Chapter 4, untreated WPI emulsions prepared using a whey protein concentration of ~ 11 g L^{-1} resulted in d_{43} values of ~ 0.6 and ~ 1.2 μm for the parent and redispersed emulsions respectively. However, the d_{43} values of the redispersed emulsions (5.0%, w/w, WPI) were 10 times greater for emulsions prepared using preheat-treated solutions containing 10% (w/w) WPI. It appears that large-scale re-coalescence in

these emulsions possibly occurred because of the presence of a vast proportion (~ 80%) of large sized aggregated proteins. It is possible that, during spray drying, as the oil droplets become compacted and are forced against each other because of the evacuation of water from the drying emulsion droplet, large sized aggregates either on the surface of the oil droplets or in the bulk phase of the emulsion droplet could potentially rupture the stabilizing film on the surface of the oil droplets, leading to the nucleation of a pore and causing oil droplet re-coalescence either at the conclusion of the spray drying process or during the redispersion of the powder in water.

The results for the parent HE emulsions showed no change in the d_{43} values upon heating above 70ºC, suggesting that hydrophobic interactions between adsorbed protein molecules on the surfaces of different oil droplets did not occur (Fig. 6.6). Some studies have reported an increase in the average droplet diameter as a result of droplet flocculation because of increased surface hydrophobicity following the heat-induced aggregation of whey proteins (Sliwinski et al., 2003; Dickinson & Parkinson, 2004; Keowmaneechai & McClements, 2006; Surh, Ward & McClements, 2006). However, other studies have reported no change in the average droplet diameter in whey-protein-stabilized emulsions heated above 60ºC (Hunt & Dalgleish; 1994; Ye, 2010).

When these emulsions were spray dried and redispersed, a shift in the droplet diameter at both concentrations of protein in the emulsions as a result of heat treatment was noted. However, the shift was only slight in the emulsion containing 0.5% (w/w) WPI (Fig. 6.6). This was expected as small sized emulsion droplets had already been formed and a limited amount of monomeric α-la and β-lg would be

present in the bulk phase of the emulsion during the heat treatment. This was indeed seen in the SDS-PAGE results, with ~ 60% of monomeric α-la and ~ 40% of monomeric β-lg still remaining in the aqueous phase of the emulsion after the heat treatment at 90ºC (Figs. 6.8 and 6.9). Also, the aggregates formed as a result of the heating were in the size range 70–250 kDa (Fig. 6.8). Because most of the monomeric α-la and β-lg was still present in the bulk phase of the emulsions after the heat treatment and because of the small proportion of relatively small sized protein aggregates, the desorption of the adsorbed monomeric protein molecules was comparable with that of the untreated emulsions at a similar concentration of protein in the emulsion. Hence, the shift in the d_{43} values was only slight in the heat-treated emulsions containing 0.5% (w/w) WPI compared with the untreated emulsions.

Based on previously collected data on unadsorbed protein in the bulk phase of WPI-stabilized emulsions (Chapter 4) and the SDS-PAGE results shown in Fig. 6.8, the residual monomeric α-la and β-lg in the bulk phase of an emulsion containing 3.0% (w/w) WPI that was preheat treated above 70ºC could be estimated to be about twice that of an emulsion containing 0.5% (w/w) WPI and preheat treated at a similar temperature. However, the d_{43} values at corresponding preheat treatment temperatures were greater in redispersed emulsions containing 3.0% (w/w) WPI than in redispersed emulsions containing 0.5% (w/w) WPI. This could also have been due to the presence of larger sized aggregates in the bulk phase of the emulsion, which may have led to the formation of “cracks” or “pores” in the interfacial layer of the emulsion droplet as a result of compaction once all the water had been evacuated out of the emulsion droplet (Fig. 6.8). Heating at 90ºC as a function of heating time showed a similar concentration-dependent shift of the

droplet size towards the larger size range in the redispersed emulsions to that seen in the emulsions in Section 6.4.1. There was also a correlation between the residual monomeric proteins and the change in d_{43} values (Figs. 6.10 and 6.11).

On closer examination of the electron micrographs, there were no major noticeable differences between untreated and heat-treated emulsions and powders. In both untreated and heat-treated powder particles, some oil droplets were seen entering and opening up at the air–water interface on the inside surface of the powder particles (Fig. 6.12g). It is possible that, on redispersion, these "free oil droplets", on the inside surface as well the outside surface of the powder particles, could coalesce to form bigger droplets and cause the size distribution to shift towards the large size range (Figs. 6.12e and 6.12g). Some semi-coalesced particles with well-formed necks were also observed in heat-treated powders; they could also have originated as a result of the presence of aggregated particles in the bulk phase of the drying droplet.

6.6 Conclusions

It is evident from these experiments that the state of the whey protein used as an emulsifier has a huge impact on the stability of the oil droplets during spray drying. Heat treatment above 70°C caused denaturation and aggregation of the whey proteins and resulted in a shift in the average droplet diameter towards larger values because of re-coalescence during drying. The depletion of monomeric species and the shift in the average droplet diameter correlated positively at lower protein concentrations (both PW and HE). The main reason for the shift in droplet diameter was probably a decrease in concentration of monomeric protein species as a result of heat-induced aggregation. A lower concentration of monomeric protein species

in the bulk phase has been suggested to lead to desorption of the monomeric whey proteins (because of their higher surface activity) from the surface of the oil droplet during drying, leaving “gaps” and consequently resulting in coalescence. No dependence of residual monomeric protein on coalescence was observed in the PW and HE emulsions containing 5.0% (w/w) and 3.0% (w/w) protein respectively. The observed changes in these emulsions can best be explained by the size of the resulting aggregates, which destabilize the oil droplets during drying. However, further study is required to better understand the destabilization mechanisms in play at these higher concentrations.

Chapter 7

Influence of heat treatment on the stability, during spray drying, of oil-in-water emulsions made using mixtures of whey protein isolate and sodium caseinate

7.1 Abstract

Emulsions of soybean oil in solutions of whey protein isolate (WPI), sodium caseinate (NaCas) and maltodextrin were prepared, heat-treated and spray dried to yield powders with ~ 55% (w/w, dry basis) oil content. The effects of heat treatment and the addition of NaCas on the stability of the emulsions were investigated by examining the particle size of the emulsions before and after spray drying, the composition of the surface of the oil droplets before and after heating and the morphology of the emulsion droplets. Previous chapters have shown that emulsions stabilized with 3.0% (w/w) protein are able to provide adequate stability to the oil droplets during the spray drying process. With heat treatment, the emulsion droplets noticeably coalesced in a whey-protein-only-stabilized emulsion at a protein concentration of 3.0% (w/w) in the emulsion. The amount of adsorbed casein changed with the heat treatment in some but not all emulsions. The emulsion droplet size was least affected when a third of the total protein in the emulsion was

substituted with NaCas, compared with emulsions containing whey protein only (no NaCas). The stability of the emulsion was noticeably improved even when NaCas was added after the homogenization step. We believe that the steric effect of caseins prevented large-scale aggregation of the whey proteins. This effect can be translated into ~ > 1.0% of unadsorbed net protein in the bulk phase of the emulsions, which provided stability to the drying droplets by migrating to the air/water interface without putting large stresses on the protein molecules present on the surface of the oil droplet.

7.2 Introduction

A stable emulsion can be defined as an emulsion that exhibits no change in droplet size distribution over the time scale of measurement. However, the time scale of measurement may vary from hours to even months, depending on the materials used to prepare the emulsion (Vega and Roos, 2006). Milk protein products have excellent emulsion-stabilizing properties, which have been well documented (Ye, 2008). An emulsified food ingredient in a powdered format offers numerous advantages from a commercial point of view, such as increased shelf life, easier handling and reduced transportation costs. Spray drying to produce such powders involves converting the ingredient, usually in a liquid form, i.e. an emulsion, into a fine mist through a process called atomization. When this fine mist is exposed to hot air, rapid evaporation of water occurs, resulting in a powder (Vega & Roos, 2006). The objective of spray drying is to have a product that can be reconstituted to a similar state to that of the parent or original system (Sliwinksi et al., 2003a).

However, coalescence of emulsion droplets has been reported when emulsions are spray dried; for milk-protein-stabilized emulsions, this causes a shift in the average droplet diameter towards a larger size range upon redispersion of the powder in water (Hogan et al., 2001; Jafari et al., 2008; Chapter 4). Chapters 4 and 5 describe the effects of spray drying on the stability of the oil droplets in emulsions prepared using non-aggregated [whey protein isolate (WPI) or sodium caseinate (NaCas)] and aggregated [calcium caseinate (CaCas) or milk protein concentrate (MPC)] proteins. Emulsions stabilized with WPI or NaCas showed no change in their size distributions upon redispersion, compared with before drying (parent emulsions) when the total protein concentration was higher than a critical value of unadsorbed protein (i.e. ~ > 1.0%, w/w) in the bulk phase of the emulsion before drying.

Typically, this optimum concentration of unadsorbed protein was ≥ 3.0% (w/w). Below this critical level of unadsorbed protein, emulsions made with both WPI and NaCas showed a shift in the droplet size distribution towards larger values. However, compared with emulsions containing WPI or NaCas, higher concentrations of MPC or CaCas in emulsions were required because of the presence of aggregated species in these protein products.

Heating β-lactoglobulin above 60°C leads to denaturation, exposing the reactive free thiol groups. Once exposed, these free thiol groups react via disulphide bonding to form oligomers or polymers, depending on the concentration of protein in solution, the heating time and temperature, the pH and the ionic strength of the solution (Damodaran & Anand, 1997; Demetriades et al., 1997; Dickinson & Parkinson, 2004; Fang & Dalgleish, 1998; Sliwinski et al., 2003b). However, α-lactalbumin does not contain any free thiol groups, but is still involved in the polymerization process (Loveday et al., 2014; Nicolai et al., 2011; Singh & Ye, 2009). Studies have shown that heat treatment of whey proteins causes a significant loss in their emulsifying ability. This has been suggested to be due to the formation of large aggregates, which are unable to cover the surface of the oil droplet efficiently, leading to coalescence (Millqvist-Fureby et al., 2001).

The effect of heat treatment above 60°C on whey-protein-stabilized emulsions has also been studied. Dickinson and Parkinson (2004) reported a tenfold shift in droplet diameters (d_{43}) towards larger values when β-lactoglobulin-stabilized emulsions (2.0%, w/w) were heated above 85°C for a prolonged period. Other studies have also reported an increase in volume-weighted mean particle diameter values (d_{43}), caused by the heat treatment of whey-protein-stabilized emulsions

(Euston et al., 2000; Keowmaneechai & McClements, 2006; Sliwinski et al., 2003b; Surh et al., 2006). This increase in the droplet size has been attributed mainly to the interactions between adsorbed protein molecules on the surfaces of different droplets, as a result of the denaturation of the whey proteins. However, other studies have reported no change in the droplet diameter values when emulsions made using different concentrations of whey proteins have been heated (Hunt & Dalgleish 1994; Ye, 2008).

In Chapter 6, the effects of heat on whey-protein-stabilized emulsions at various concentrations before and after spray drying as a function of heating time and temperature were studied. The results showed no change in the droplet size in whey-protein-stabilized emulsions heated between 60 and 90°C. Upon spray drying and redispersion, these emulsions showed comparatively larger d_{43} values, relative to those in the untreated redispersed emulsions. This shift in droplet size distribution was thought to be due to the denaturation and aggregation of whey protein because of the heat treatment, resulting in a decrease in the residual monomeric whey protein species that are required to stabilize emulsions during the drying process.

Bovine casein comprises four types of protein, namely α_{s1}-, α_{s2}-, β- and κ-caseins. α_{S1}- Casein and β-casein do not contain any cysteine residues. In contrast, α_{s2}-casein and κ-casein each contain two cysteine residues and are susceptible to polymerization. It has been reported that large-scale aggregation of whey proteins is inhibited in the presence of a small amount of caseinate (Dickinson & Parkinson, 2004). This is thought to be due to the steric stabilization provided by the small amount of adsorbed casein molecules (especially β-casein).

In this chapter the previous work relating to the heat treatment of whey-protein-stabilized emulsions has been extended to examine the influence of the presence of NaCas on the stability of such emulsions during spray drying.

7.3 Materials and methods

Whey protein isolate (WPI), sodium caseinate (NaCas), soybean oil and maltodextrin (MD) were used in this study. The details of these materials and the equipment used in the study have been described in Chapter 3 (section 3.1 and 3.2). Figure 7.1 a and b show the process flow diagrams of emulsion preparation, spray drying and analyses for set 1 and set 2. Details of emulsion preparation for set 1 and set 2 have been described in section 7.3.1 and 7.3.2.

7.3.1 Emulsion preparation and heat treatment – set 1

Proteins (WPI or/and NaCas) were dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solution was stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solution was then adjusted to 7.0 using 1M HCl or 1M NaOH if required. Similarly, MD solution was prepared using the same stirring equipment with stirring until the powder was dissolved completely. Soybean oil at 20.0% (w/w) was blended with the aqueous solutions of protein (WPI or/and NaCas) and MD using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Formulations used for emulsion preparation have been shown in table 7.1.

Approximately, 500 mL of each emulsion variant was dispensed into glass bottles (Schott AG, Mainz, Germany) and heat-treated to 90ºC by introducing the glass bottles in a steam-jacketed kettle with boiling water. A digital thermometer with thermocouple wire was used to record the temperature of the emulsion. While the emulsion was being heated, the bottles were being gently swirled for efficient heat distribution in the emulsion. As soon as the temperature of the emulsion reached 90ºC (3–4 min), the emulsions were immediately transferred to another water bath pre-set at 90 ºC. The emulsions were held for 10 min in the water bath. Following this, the emulsions were cooled in an ice bath to room temperature and refrigerated until these were spray dried (Fig 7.1 a).

7.3.2 Emulsion preparation and heat treatment – set 2

Proteins (WPI and NaCas) were dissolved in water purified by reverse osmosis (RO) using an IKAMAG RET stirrer (Basic C, IKA-Werke, Staufen, Germany) and the solution was stirred at 300 rpm for at least 6 h at room temperature to permit complete hydration. The pH of the protein solution was then adjusted to 7.0 using 1M HCl or 1M NaOH if required. Similarly, MD solution was prepared using the same stirring equipment with stirring until the powder was dissolved completely. Soybean oil at 20.0% (w/w) was blended with the aqueous solution of protein (WPI only) and MD using an Ultra-Turrax T-25 hand-held homogenizer (Janke & Kunkel, Staufen, Germany) operated at 13,500 rev min^{-1} for 2 min. The resulting primary emulsion was further homogenized at 60 MPa for one cycle using a microfluidizer (M-110P, Microfluidics, Newton, MA, USA). Once homogenised, NaCas solution was added to the emulsions and the mixture was stirrer for 5 min at 300 rpm using

the same stirring equipment. Formulations used for emulsion preparation have been shown in table 7.1.

Approximately, 500 mL of each emulsion variant was dispensed into glass bottles (Schott AG, Mainz, Germany) and heat-treated to 90 ºC by introducing the Schott bottle into a steam-jacketed kettle with boiling water. A thermometer with thermocouple wire was used to record the temperature of the emulsion. While the emulsion was being heated, the bottles were being gently swirled for efficient heat distribution in the emulsion. As soon as the temperature of the emulsion reached 90 ºC (3–4 min), the emulsions were immediately transferred to another water bath pre-set at 90 ºC. The emulsions were held for 10 min in the water bath. Following this, the emulsions were cooled in an ice bath to room temperature and refrigerated until these were spray dried (Fig 7.1 b).

Table 7.1 *Formulation of set 1 and set 2 emulsions used in this study.*

WPI (%)	NaCas (%)	Oil (%)	MD (%)	Water (%)
3.0	0.0	20.0	12.0	65
2.9	0.1	20.0	12.0	65
2.75	0.25	20.0	12.0	65
2.5	0.5	20.0	12.0	65
2.25	0.75	20.0	12.0	65
2.0	1.0	20.0	12.0	65

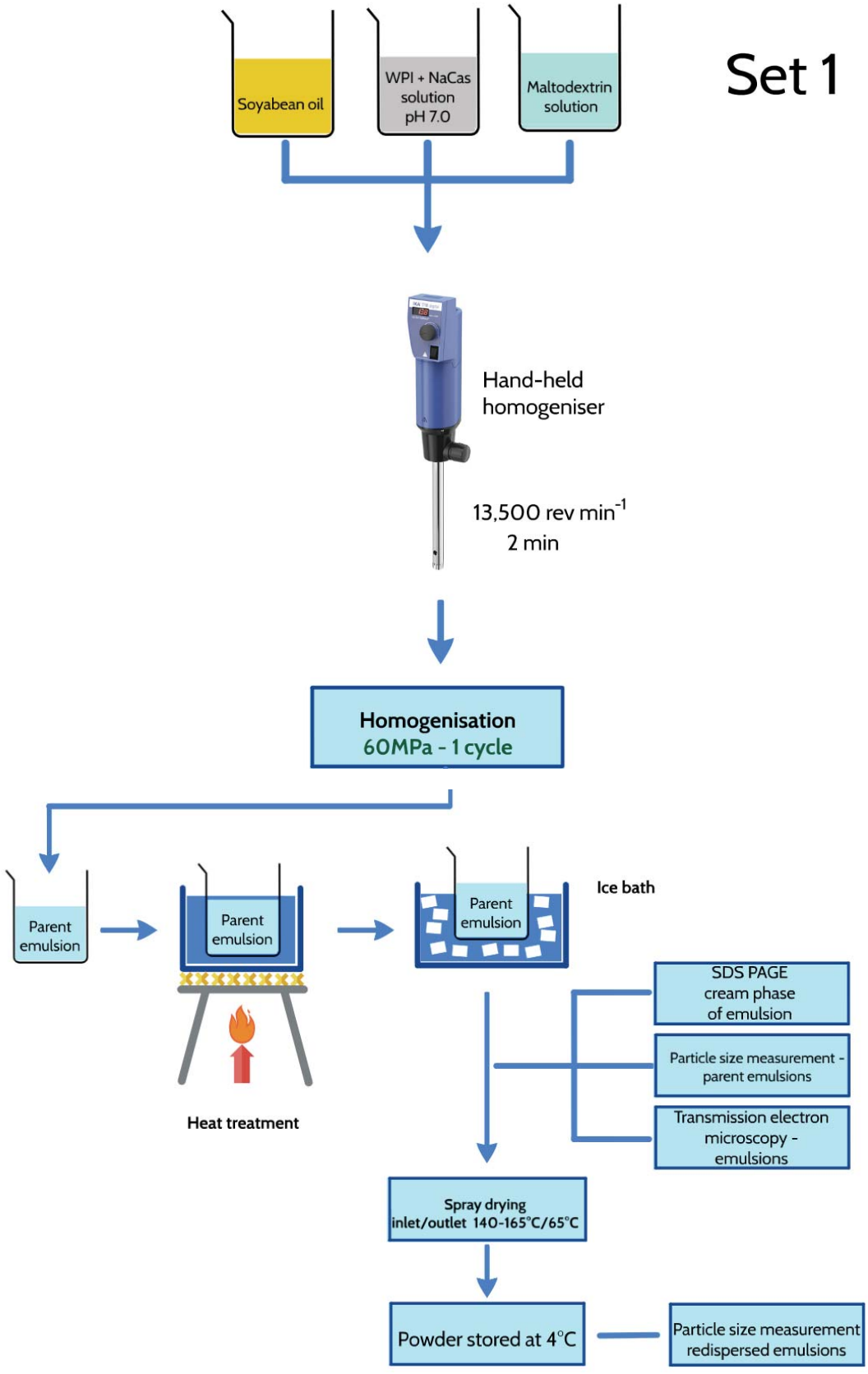


Figure 7.1a *Process flow diagram showing the emulsion preparation, spray drying and analysis for set 1 in this study.*

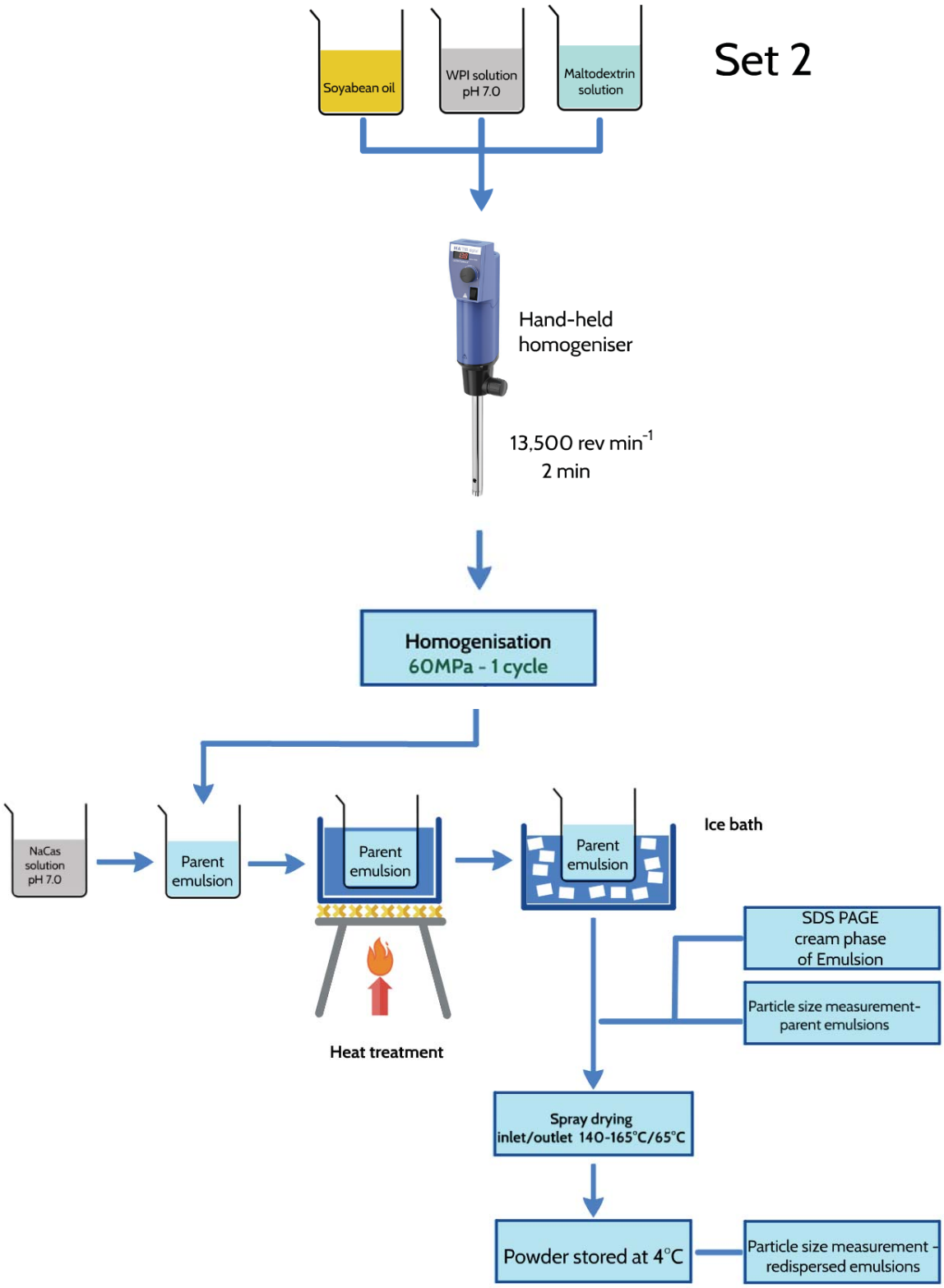


Figure 7.1b *Process flow showing the emulsion preparation, spray drying and analyses for set 2 in this study.*

7.3.3 Spray drying

All emulsions were spray dried using a Buchi bench-top spray drier (B-290, Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current configuration and fitted with a two-fluid nozzle (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The inlet temperature varied between 140 and 165 °C and the outlet temperature was 65 °C. The resulting powders were collected in airtight containers, allowed to cool to ambient temperature and then stored at 4 °C for further analysis.

7.3.4 Determination of average droplet diameter

The diameters of the oil droplets for both the parent emulsions (fresh emulsions which have not been spray dried) and the redispersed emulsions (emulsions prepared by dissolving powder in RO water) were determined by the laser light scattering method as described by Jafari et al. (2007) and Ye (2011) using a Mastersizer E (Malvern Instruments, Malvern, Worcestershire, UK). Redispersed emulsions were prepared by reconstituting the dried emulsion powder in RO water to obtain the same total solids content as the parent emulsion. Powder was slowly added to RO water and the dispersions were stirred for at least 30 min before particle size measurements were made. In some cases, the emulsions were diluted 1:1 with 2.0% (w/w) sodium dodecyl sulphate (SDS) solution before the measurements were made. The comparison of the size distributions in RO water alone and with the addition of SDS indicated flocculation/aggregation during emulsification/redispersion (Sanchez & Patino, 2005).

7.3.5 Polyacrylamide gel electrophoresis (PAGE)

The extent of aggregation of both the adsorbed protein and the non-adsorbed protein in the aqueous phase of preheated emulsions was determined using SDS polyacrylamide gel electrophoresis (SDS-PAGE), as described by Ye & Singh (2000). For non-reduced gels, a certain amount of emulsion was mixed with SDS buffer (0.5 M Tris, 2.0% SDS, pH 6.8), which was then centrifuged (13,000 *g* for 10 min, Sorvall RC-5C, DuPont Co., Wilmington, DE, USA) to separate the cream layer. For reduced gels, SDS buffer, containing 0.05% 2-mercaptoethanol, was added to emulsions, which were heated to 95ºC for 5 min and centrifuged as above. The samples for both types of gel were cooled to room temperature and a portion (20 μL) of the subnatant was applied to SDS gels (Criterion Precast Gels, 10–20% Tris-Tricine, Bio-Rad Laboratories, Hercules, CA, USA) at 150 V using a power supply unit (PowerPac Basic, Bio-Rad Laboratories, Richmond, CA, USA). Preheated WPI solutions were also mixed with the two SDS buffers in the same way as the heated emulsions and a portion (20 μL) of the dispersion were applied to SDS gels as described above. All gels were then stained with Coomassie Blue R-250 (0.05%, w/v, in 25%, v/v, isopropanol, 10%, v/v, acetic acid) for 30 min and then destained using a destaining solution (10%, v/v, isopropanol, 10%, v/v, acetic acid). These gels were scanned using a gel imaging system (Gel Doc System, Bio-Rad Laboratories, Richmond, CA, USA). The gels were analysed for the density of bands using the volume tool in Image Lab software (Bio-Rad Laboratories, Richmond, CA, USA).

7.3.6 Data analysis

Each data point represents average value of two determinations on two separate emulsions (measured in duplicates). Error bars in graphs represent standard deviation of average values of two determinations on separate emulsions. Some graphs were drawn using Sigmaplot 13 graphing and data analysis software (Systat Software Inc., San Jose, CA, USA) and other were drawn using Microsoft Excel 2011 (Microsoft Corporation, WA, USA).

7.4 Results

Emulsification of soybean oil in a solution of either WPI or a combination of WPI and NaCas with MD resulted in parent emulsions (Set 1) having monomodal and narrow droplet size distributions (Fig. 7.2). There was no change in size distribution with the addition of 0.1 to 1.0% (w/w) NaCas to the emulsions, with a concomitant decrease in the WPI concentration from 3.0 to 2.0% (w/w, Figs. 7.2a–7.2h). This was consistent with the findings in Chapter 4; that is, emulsions made with WPI or NaCas showed very similar average droplet diameter values above a total protein concentration of > 2.0% in the emulsions. In this study, the net concentration of protein in all emulsions was at 3.0% (w/w) and a heat treatment at 90°C for 10 min was used.

Upon heat treatment of the parent emulsions at 90°C for 10 min, no change in the average droplet diameter was observed, regardless of the concentration of NaCas in the emulsion (Fig. 7.3). The untreated emulsion containing 3.0% (w/w) WPI remained stable during spray drying, as the average droplet diameter showed very little shift upon spray drying and redispersion (Fig. 7.2a). This was consistent with

the results from Chapter 6. This was also observed in untreated emulsions containing a combination of WPI (2.0%, w/w) and NaCas (1.0%, w/w) (Fig. 7.2b).

In the case of the emulsion containing 3.0% WPI (w/w) only, a marked change in the size distribution upon spray drying as a result of heat treatment of the parent emulsion at 90°C for 10 min was observed. In these emulsions, the distribution changed from being narrow and monomodal, with a relatively small size range, to being bimodal, with an additional peak at ~ 10 μm (Fig. 7.2c). With the replacement of 0.1% (w/w) WPI with NaCas in the emulsion, a shift in the droplet size distribution towards the smaller size range was seen (Fig. 7.2d), compared with the heat-treated redispersed emulsion containing 3.0% (w/w) WPI alone. The proportion of droplets under the second peak (~ 10 μm) decreased noticeably with the addition of NaCas to the parent emulsion. With further increases in the concentration of NaCas from 0.1 to 1.0% (w/w), the droplet size distributions of the redispersed emulsions became narrower and moved towards those of the respective parent emulsions (Figs. 7.2e–7.2h). At concentrations of 1.0% (w/w) NaCas and 2.0% (w/w) WPI in the emulsion, there was a small proportion of particles between 1 and 10 μm, but the majority of the other particles were in the same size range as for the parent emulsion (Fig. 7.2h). Upon dilution (1:1) of the emulsions with SDS solution, there was no noticeable change in the size distribution. Therefore, we can infer that the primary cause of the change in the distribution upon the addition of NaCas and the subsequent heat treatment was droplet re-coalescence (Figs. 7.2c–7.2h).

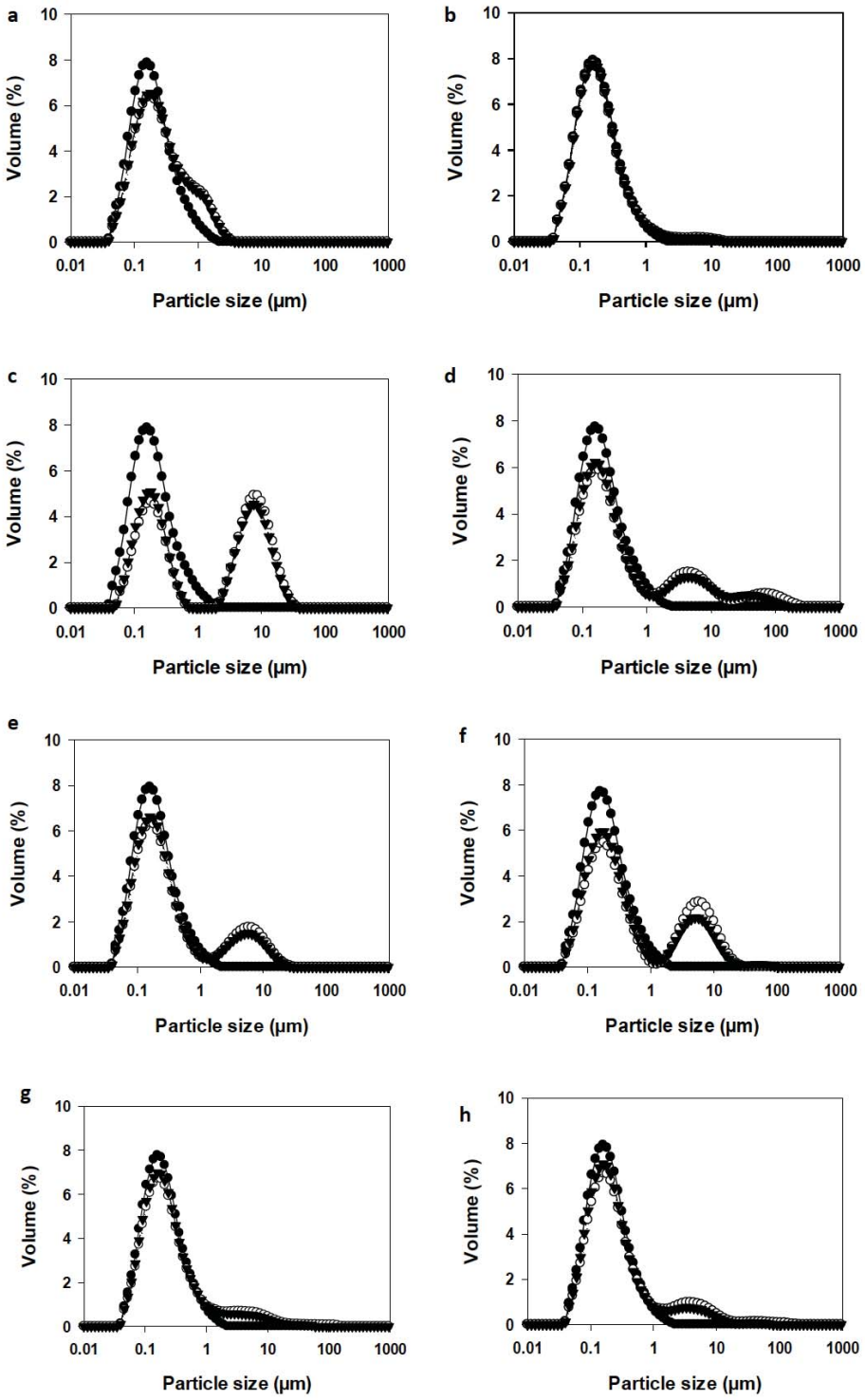


Figure 7.2 *Droplet size distributions of emulsions containing 2.0–3.0% (w/w) whey protein isolate, 0.0–1.0% (w/w) sodium caseinate, 20.0% (w/w) soybean oil and 12.0% (w/w) maltodextrin before spray drying (●) and after redispersion (○). These emulsions were heat treated at 90°C for 10 min before spray drying (except a and b). All emulsions were also diluted (1:1) with 2.0% (w/w) sodium dodecyl sulphate solution (▼). The whey protein isolate and sodium caseinate concentrations respectively were: a, 3.0% and 0.0% (unheated); b, 2.0% and 1.0% (unheated); c, 3.0% and 0.0%; d, 2.9% and 0.1%; e, 2.75% and 0.25%; f, 2.5% and 0.5%; g, 2.25% and 0.75%; h, 2.0% and 1.0%.*

In agreement with the particle size distribution data (Fig. 7.2), the d_{43} values decreased (slightly but variably) with increasing NaCas concentration from 0.1 to 1.0% (w/w) in the emulsions (Fig. 7.3). This indicated that the majority of the shift in the distributions in the heat-treated emulsions post spray drying was due to the re-coalescence of oil droplets. The emulsion stability seemed to improve considerably with the addition of 1.0% (w/w) NaCas, as the d_{43} value of the redispersed emulsion was close to ~ 1 μm, which was only slightly larger than that of untreated emulsions containing 3.0% (w/w) WPI, which was measured as being ~ 0.5 μm (Fig. 7.3).

The SDS-PAGE patterns of the cream phase obtained from the unheated parent emulsions are shown in Fig. 7.4a. Proteins, that were present in the aqueous phase of the emulsions and were not associated with the oil droplet surface, were removed by centrifugation and subsequent washing of the cream. Casein proteins were absent from the emulsion made using WPI only, as expected (Fig. 7.4a, lane 1). However, the β-casein band was clearly visible with the addition of 0.25% (w/w) NaCas to the emulsion (Fig. 7.4a, lane 2) and all casein bands were clearly present with the further addition of ≥ 0.5% (w/w) NaCas (Fig. 7.4a, lanes 3–5).

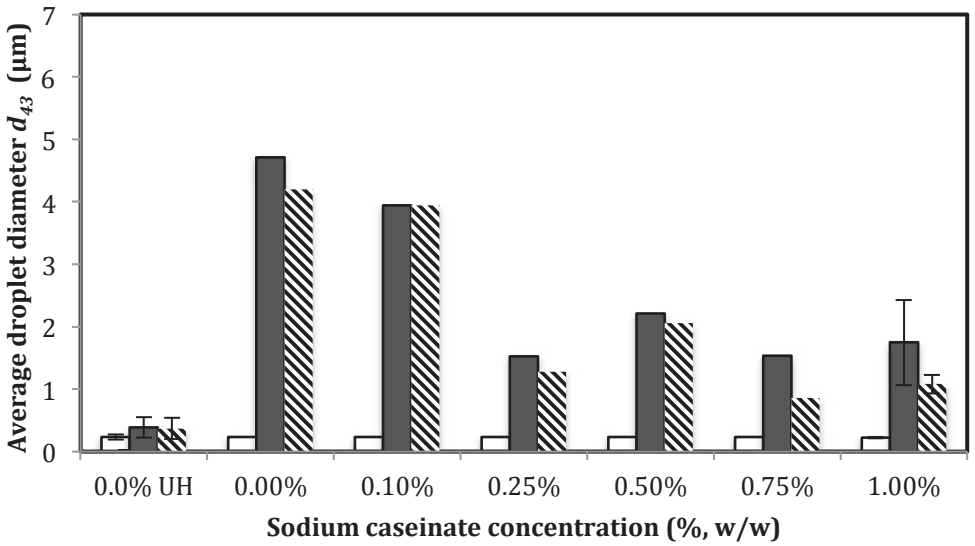


Figure 7.3 *Average droplet diameter (d_{43}) values of parent (blank bars) and spray dried and redispersed (solid bars) emulsions containing 20.0% (w/w) soybean oil, 12.0% (w/w) maltodextrin and 2.0–3.0% (w/w) whey protein isolate and 0.0–1.0% (w/w) sodium caseinate. The total concentration of protein in all emulsions was kept constant at 3.0% (w/w). The redispersed emulsions were also diluted (1:1) with 2.0% (w/w) sodium dodecyl sulphate solution (striped bars). UH denotes unheated emulsion.*

The electrophoretic pattern of the 3.0% (w/w) WPI emulsion after heat treatment showed some protein polymerization (Fig. 7.4b, lane 1), indicated by two noticeable bands corresponding to a molecular weight between those of α_{s2}-casein and bovine serum albumin. These bands were only faintly visible in the unheated emulsions (Fig. 7.4a, lane 1). In addition, a little aggregated material appeared to be present at the entrance of the stacking gels after heating the emulsions containing whey protein (Fig. 7.4, lane 1). The most prominent band, labeled as 'X', had

slightly lower mobility than the casein bands. Based on its estimated molecular weight (~ 38 kDa), this band was likely to be dimeric β-lactoglobulin (Havea et al., 2001). The band labelled 'Y' was likely to be tri-meric β-lactoglobulin (Havea et al., 2001). Upon heat treatment of the emulsions containing whey and casein proteins, β- and κ-caseins were observed at NaCas concentrations ≥ 0.25%. As the concentration of NaCas in the emulsions was increased, the bands labeled X and Y decreased in intensity compared with these bands in the heat-treated whey-protein-only emulsion. There was no noticeable change in the intensities of the β-lactoglobulin (β-lg), α-lactalbumin (α-la) and bovine serum albumin (BSA) bands in the heat-treated emulsions (Fig. 7.4b). These results raised the following question. Would the addition of NaCas to the aqueous phase of the emulsion after homogenization have a similar effect on the stability of the emulsions during drying?

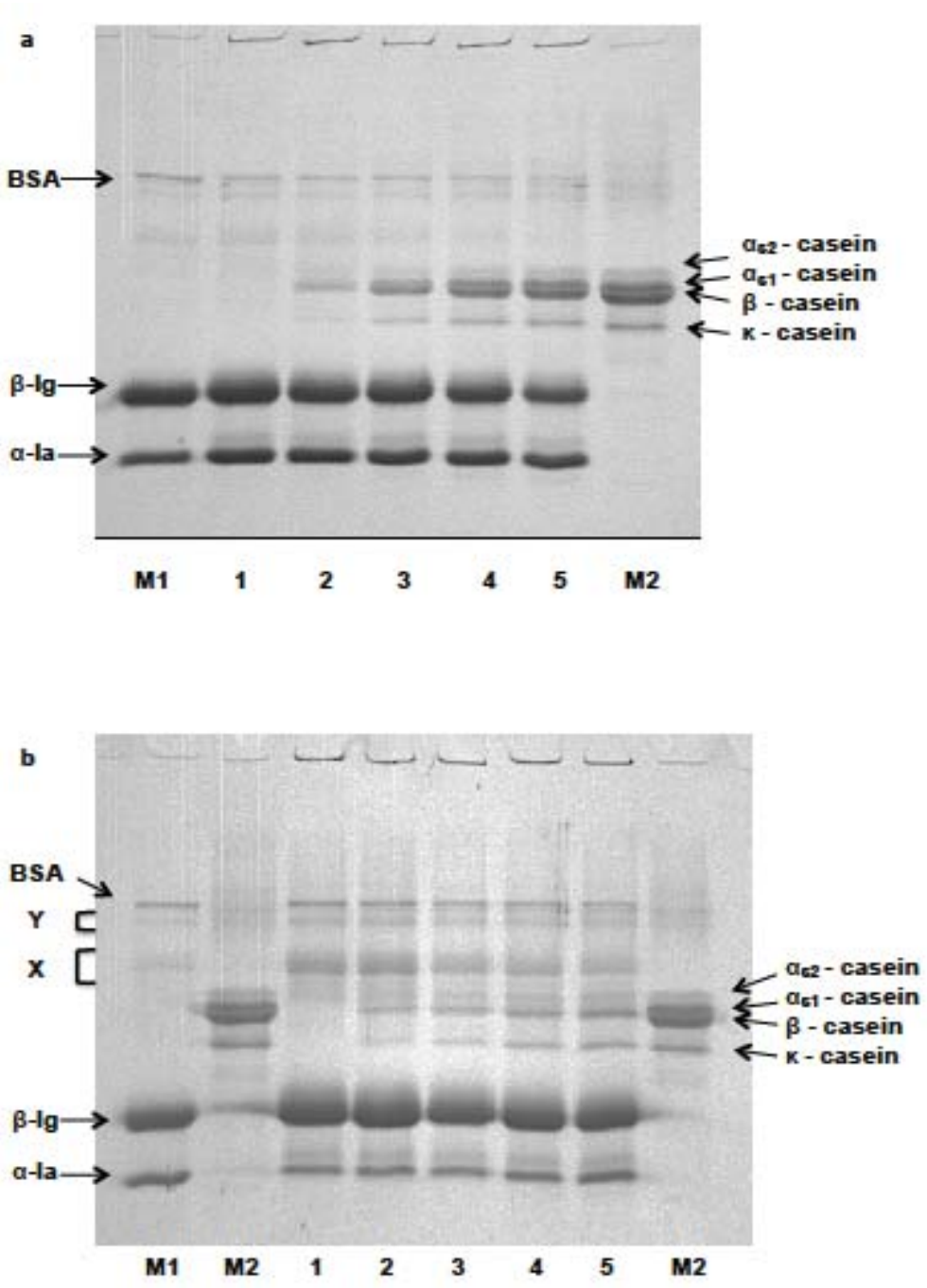


Figure 7.4 *SDS-PAGE patterns of the cream phase of emulsions (non-reducing) formed with a combination of whey protein isolate and sodium caseinate (a) before and (b) after heating at 90°C for 10 min. Lane 1: 3.0% WPI, 0.0% NaCas; lane 2: 2.75% WPI, 0.25% NaCas; lane 3: 2.5% WPI, 0.5% NaCas; lane 4: 2.25% WPI, 0.75% NaCas; lane 5: 2.0% WPI, 1.0% NaCas. M1 denotes unheated WPI, M2 denotes unheated NaCas and BSA denotes bovine serum albumin.*

Therefore, another set of emulsions (Set 2), in which a NaCas solution was added to the WPI emulsion after homogenization but before the heat treatment, was prepared. The total protein concentration was maintained at 3.0% (w/w) in all emulsions. The particles sizes were measured after the NaCas solutions had been added to the parent WPI emulsions (pre-homogenized). There was no noticeable change in the d_{43} values of the parent emulsions with an increase in the concentration of NaCas in the emulsion from 0.1 to 1.0% (w/w) (Fig. 7.5). There was also no noticeable change in the d_{43} values of these emulsions with the application of heat at 90°C for 10 min (Fig. 7.5). The d_{43} values of the parent emulsions (Fig. 7.5) were comparable with those of the parent emulsions presented earlier (Fig. 7.3).

The d_{43} values increased markedly upon spray drying and redispersion of the 3.0% (w/w) WPI emulsions in RO water, and further increased with the addition of 0.1% (w/w) NaCas. However, the d_{43} values showed a shift towards smaller values with further increases in the NaCas concentration in the emulsions, with the values being between 1 and 2 μm with the addition of 1.0% (w/w) NaCas (Fig. 7.5). The d_{43} of the redispersed emulsions presented in Fig. 7.5 were larger, compared with those of the emulsions in which NaCas was added before homogenization (Fig. 7.3).

The SDS-PAGE patterns of the cream phase, obtained from the unheated and heat-treated emulsions (90°C for 10 min) in which NaCas was added after homogenization, are shown in Fig. 7.6. β-Casein was the only casein protein present at the droplet surface at a NaCas concentration of 0.25% (w/w) in the unheated parent emulsion (Fig. 7.6a, lane 2). Although the NaCas was added after homogenization, some caseins still adsorbed at the droplet surface. Studies have shown the tendency of caseins to partly displace whey proteins present on the

interface of oil droplets when added to washed WPI emulsions (Dalgleish et al., 2002). With an increase in the NaCas concentration in the bulk phase of the emulsions to ≥ 0.5% (w/w), bands corresponding to α_{s2}- and κ-casein were observed, albeit at very low intensities compared with those in the untreated emulsions presented in Fig. 7.4.

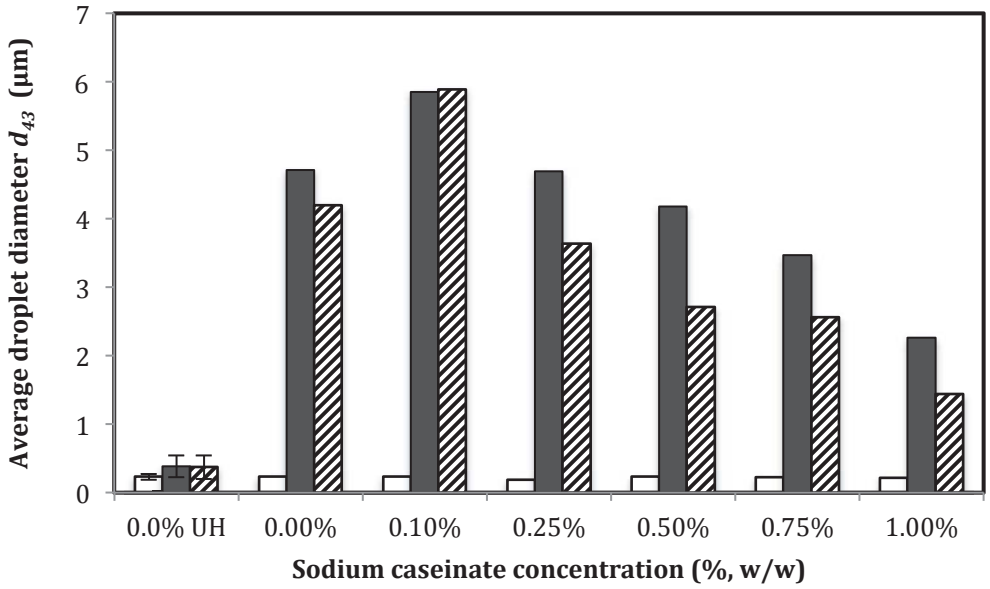


Figure 7.5 *Average droplet diameter (d_{43}) values of parent (blank) and spray-dried and redispersed (solid) emulsions containing 20.0% (w/w) soybean oil, 12.0% (w/w) maltodextrin and 2.0–3.0% (w/w) whey protein isolate and 0.0–1.0% (w/w) sodium caseinate. Sodium caseinate (in solution form) was added after homogenization of the WPI emulsions (set 2) while maintaining the total protein concentration in all emulsions at 3.0% (w/w). The redispersed emulsions were also diluted (1:1) with 2.0% (w/w) sodium dodecyl sulphate solution (striped). UH denotes unheated emulsion.*

Upon heat treatment of the emulsion containing WPI alone, polymeric species labelled ‘X’ and ‘Y’ were observed; they were likely to be dimers and trimers of β-lactoglobulin (Fig. 7.6b, lane 1). As the NaCas concentration in the emulsions was increased, the most noticeable caseins on the droplet interface were β-casein and κ-casein (Fig. 7.6b). The intensities of the bands corresponding to the likely dimers and trimers of β-lactoglobulin also reduced with an increase in the NaCas concentration in the bulk phase of the emulsions. There were no large aggregates at the entrance of the stacking gel.

TEM of redispersed emulsions with different WPI and NaCas concentrations and different treatments revealed subtle differences in particle morphology (Fig. 7.7). Dark ‘electron-dense’ particles, which were associated with the surface of the emulsion droplets, were clearly noticeable in all redispersed emulsions. These electron-dense particles were present in larger numbers in the heat-treated emulsions containing 3.0% (w/w) WPI alone than in the unheated emulsions and the heat-treated emulsion containing 1.0% (w/w) NaCas. The morphologies of these dark particles were both spherical and elongated and were present in all emulsions. Some very large dark particles that resembled a filamentous structure were also seen in the heat-treated emulsions containing 0.5% (w/w) NaCas and 2.5% (w/w) WPI (Fig. 7.7 c) The composition of these particles was not clear from the images. The emulsion droplets in the micrographs were consistent with the particle size results reported in Fig. 7.3.

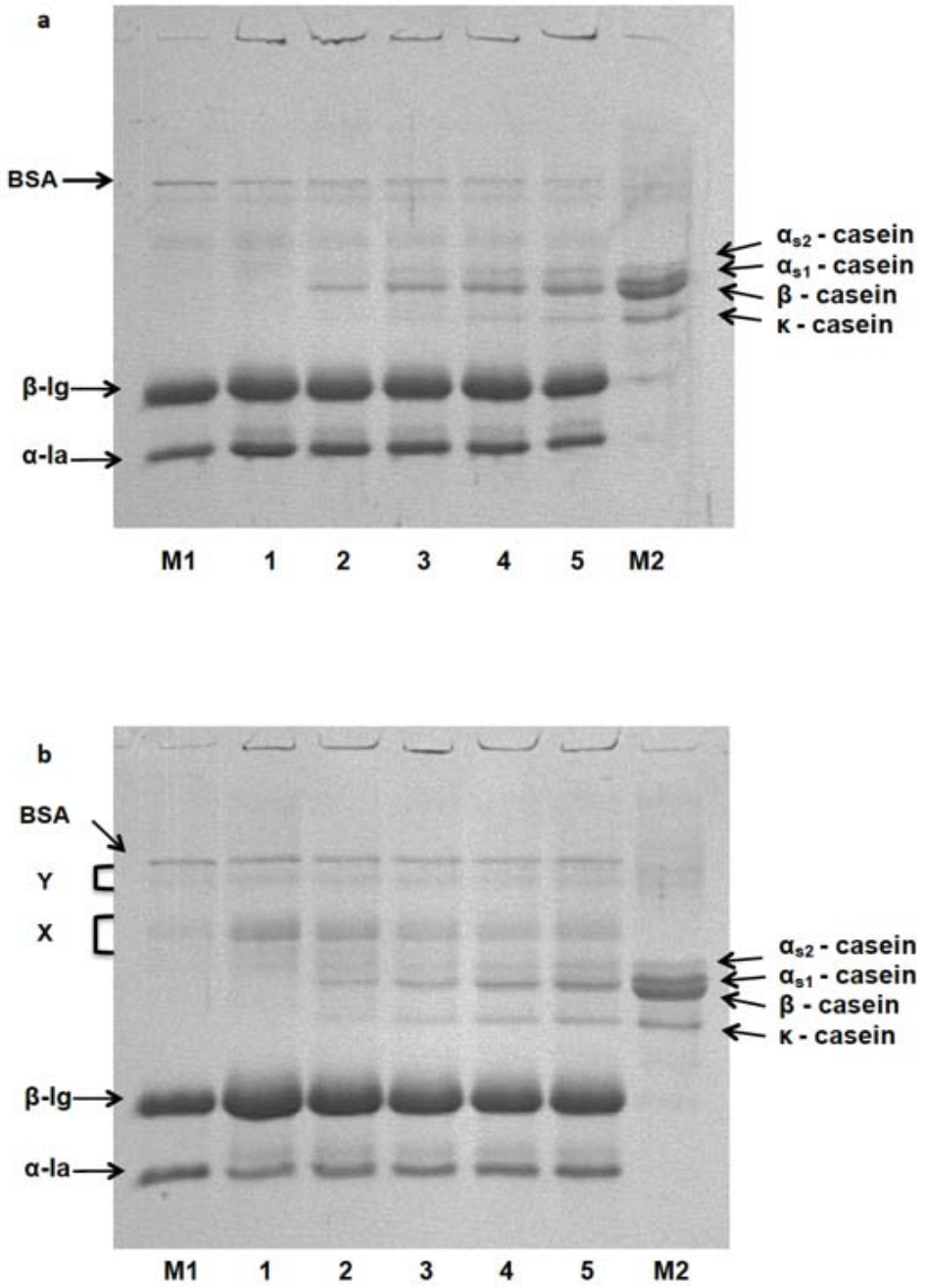
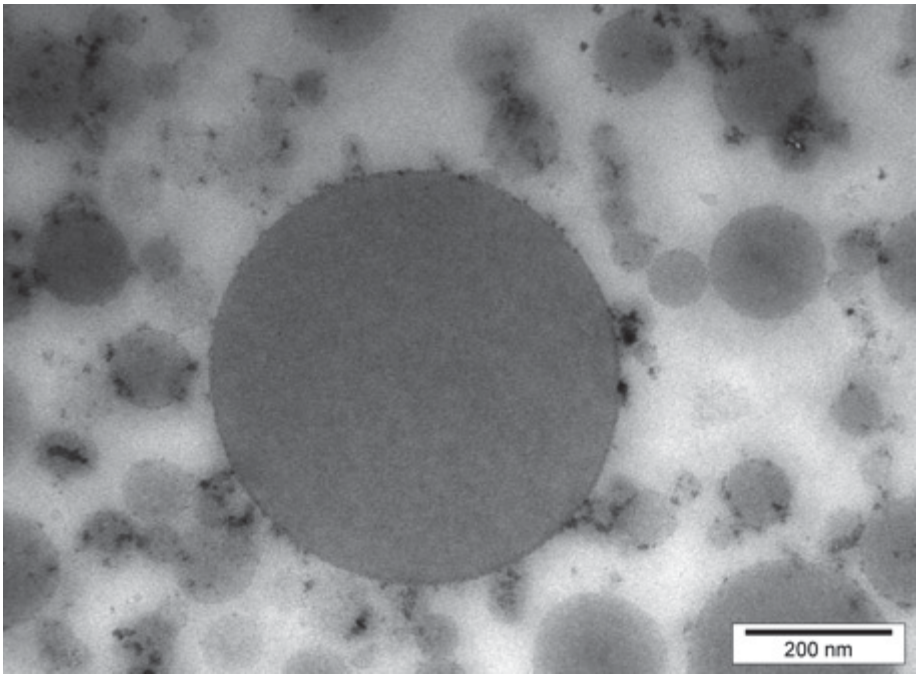
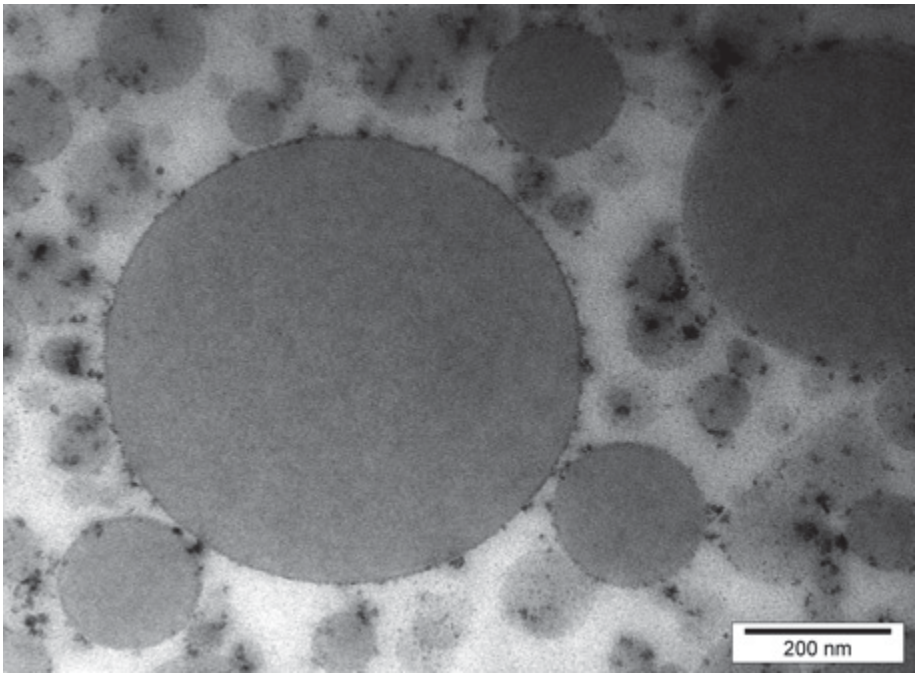


Figure 7.6 *SDS-PAGE patterns of the cream phase of emulsions (non-reducing) formed with a combination of whey protein isolate and sodium caseinate in which the sodium caseinate was added to the whey protein isolate emulsion after the homogenization step and either (a) before heat treatment or (b) after heat treatment at 90°C for 10 min. Lane 1: 3.0% WPI, 0.0% NaCas; lane 2: 2.75% WPI, 0.25% NaCas; lane 3: 2.5% WPI, 0.5% NaCas; lane 4: 2.25% WPI, 0.75% NaCas; lane 5: 2.0% WPI, 1.0% NaCas. M1 and M2 denote unheated whey protein isolate solution and sodium caseinate solution respectively and BSA denotes bovine serum albumin.*

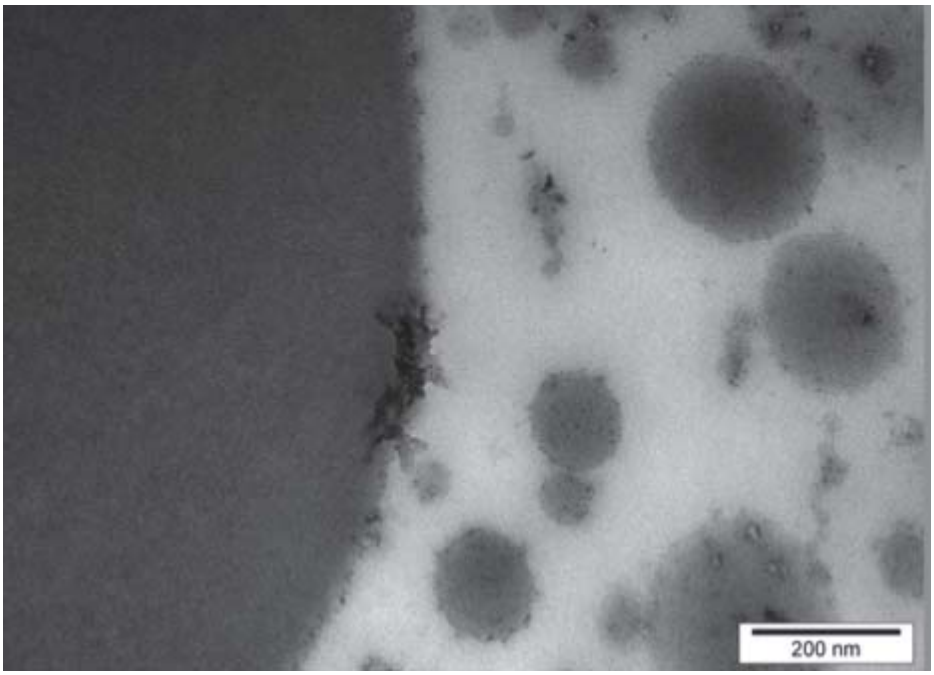
a)



b)



c)



d)

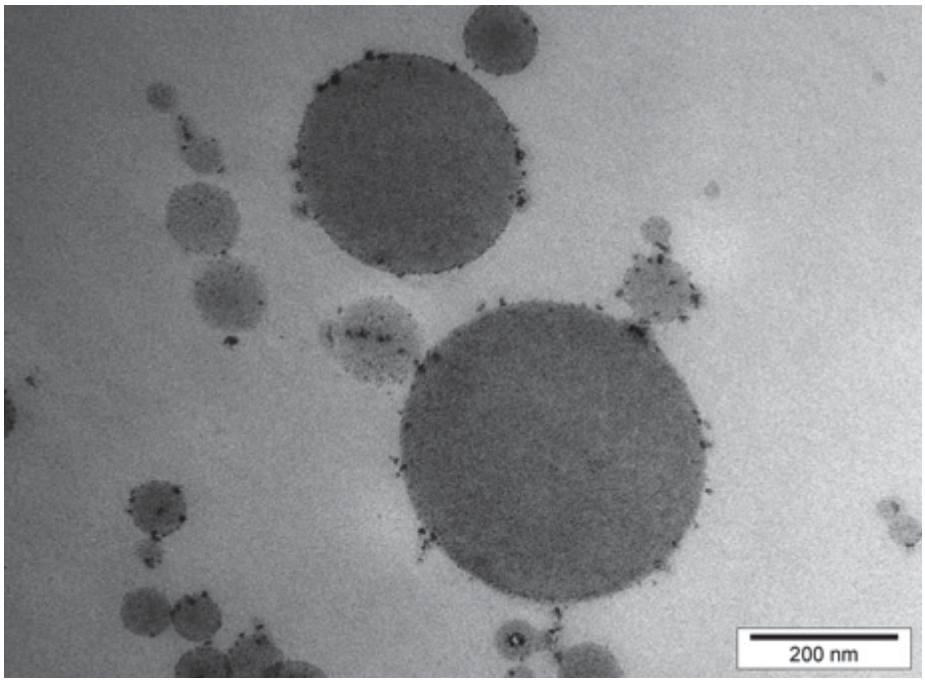


Figure 7.7 *Transmission electron micrographs of redispersed emulsions containing: (a) 3.0% (w/w) whey protein isolate, untreated; (b) 3.0% (w/w) whey protein isolate, heat treated at 90℃ for 10 min; (c) 2.5% (w/w) whey protein isolate and 0.5% (w/w) sodium caseinate heat treated at 90℃ for 10 min; (d) 2.0% (w/w) whey protein isolate and 1.0% (w/w) sodium caseinate heat treated at 90℃ for 10 min. Scale as shown in each image. Sodium caseinate was added before the homogenization step for emulsions shown in c and d (set 1).*

7.5 Discussion

The minimum whey protein concentration that is required at the interface of the oil droplet to form a stable emulsion has been reported to be ~ 1.5 mg m^{-2}. This limiting concentration is reported to be slightly lower (~ 1.0 mg m^{-2}) for NaCas-stabilized emulsions because of their highly flexible structure, compared with the rigid structure of the globular whey proteins (Hunt & Dalgleish, 1994). A small average droplet diameter and a narrow distribution are achieved at a protein concentration of 3.0% in whey-protein-stabilized parent emulsions (Euston & Hirst, 1999; Hunt & Dalgleish, 1994; Srinivasan et al., 1999, 2001; Ye, 2008). By substituting some of the whey protein with NaCas and keeping the total protein concentration constant at 3.0% (w/w), there was no noticeable change in either the average droplet diameter values or the size distributions in the untreated emulsions (Set 1). The emulsions in which NaCas was added after homogenization (Set 2) also had small droplet diameters, similar to those of the emulsions containing 3.0% (w/w) protein at the time of homogenization. This was due to the excellent ability of these milk proteins to rapidly adsorb at the newly formed interface during homogenization, preventing oil droplet re-coalescence and flocculation (Dickinson, 2008; Euston & Hirst, 1999; Hunt & Dalgleish, 1994; Singh & Ye, 2009).

Whey proteins are known to denature and aggregate via disulphide bonding with heating above 65°C. Heat treatment above this temperature leads to the whey proteins exposing their hidden hydrophobic groups and interacting with themselves, leading to the formation of aggregated polymers in whey-protein-based systems (Oldfield et al., 2000). Depending on the whey protein concentration in solution, the heat treatment may lead to aggregation, gelation or even precipitation of the

whey protein aggregates (Mahmoudi et al., 2007). Heating of WPI and heating of pure β-lactoglobulin solution result in aggregates with very similar configurations (Mahmoudi et al., 2007).

However, the denaturation and the aggregation of whey proteins once adsorbed on to the surface of oil droplets in emulsions proceed differently from those in whey protein solutions. Whey protein molecules present in the aqueous phase of emulsions can interact with each other to form larger molecular weight aggregates, depending on the concentration of unadsorbed whey protein in the aqueous phase (Dickinson & Parkinson, 2004; Euston et al., 2000). This was observed in Chapter 6 where the SDS-PAGE patterns of the aqueous phase of the heat-treated emulsion containing 3.0% (w/w) WPI showed the presence of larger sized aggregates that were unable to enter the stacking gel upon heat treatment at 90°C for 10 min. These large aggregates were also accompanied by a huge reduction in the amount of monomeric β-lactoglobulin in the same emulsions.

Aggregation between protein molecules on the surface of the oil droplets and unadsorbed proteins in the bulk phase could also occur (Euston et al., 2000). The SDS-PAGE patterns in Figs. 7.4 and 7.6 show the electrophoretic intensities of polymeric species (labelled ‘X’ and ‘Y’) in the molecular weight range 25–60 kDa in the heat-treated WPI-only emulsions; they were absent in the untreated emulsion and were likely to be covalently linked dimers and trimers of β-lactoglobulin (Fig. 7.4b).

Aggregation between whey proteins adsorbed on the surfaces of different droplets, i.e. inter-droplet aggregation, has also been suggested (Euston et al., 2000). In this case, there is more likely to be a change in the average droplet diameter because of

the formation of aggregated droplets. This phenomenon has been reported in oil-in-water emulsions stabilized with whey proteins; an increase in the average droplet diameter was observed after heat treatment between 50 and 90°C for 15 min. In addition, with an increase in the holding time, the particle size also showed a shift towards larger values (Keowmaneechai and McClements, 2006). Dickinson and Parkinson (2004) reported an increase in the d_{43} values of whey-protein-stabilized (2.0%, w/w) emulsions with heat treatment at 90°C for an extended period. The particle size shifted towards larger values when these emulsions were heated at 90°C for up to 8 h, with no further increase in the d_{43} values beyond 8 h and up to 48 h. It was suggested that this was a result of attractive inter-droplet protein interactions causing weak flocculation and increases in the apparent viscosity and the droplet diameter of the emulsions (Dickinson & Parkinson, 2004). There was no change in the average droplet diameter of the parent emulsions upon heat treatment, which ruled out the possibility of interactions (covalent or non-covalent) between whey proteins adsorbed on the surfaces of different droplets (inter-droplet aggregation) causing oil droplet aggregation/flocculation.

Dickinson and Parkinson (2004) also showed that the addition of caseins can protect the whey proteins from the adverse effects of heat treatment, even at a very low concentration of 0.3–0.15% (w/w) in a 3.0%-protein-stabilized emulsion. They found that excellent stability towards prolonged heating with no change in the d_{43} values was achieved by substituting 10% of the total whey protein present in the emulsions (3.0%, w/w, WPI) with NaCas. This was suggested to be due to the enhanced steric stabilization of the emulsion droplets because of the presence of casein (especially β-casein) in the adsorbed layer (Dickinson & Parkinson, 2004). The SDS-PAGE results (Figs. 7.4 and 7.6) in this study for both Set 1 and Set 2

confirm the presence of caseins in the adsorbed layer, even at a low NaCas addition rate of 0.25% (w/w). There was a concomitant decrease in the intensities of dimers and trimers of β-lactoglobulin post heating as a result of the addition of NaCas to both sets of emulsion, i.e. NaCas addition before and after homogenization.

However, when emulsions containing 3.0% (w/w) WPI were heated, the whey proteins in the aqueous phase of the emulsion aggregated. The impact of the addition of NaCas on the composition and the structure of the resulting aggregates in the aqueous phase of whey-protein-stabilized emulsions has not been widely reported. In recent studies, it has been suggested that some caseins (α_{s1}- and β-caseins) present in the bulk phase are able to act like chaperones, preventing the irreversible aggregation that is induced by thermal stress (Kehoe & Foegeding, 2011; O'Kennedy & Mounsey, 2006). To be classified as a chaperone, this protein must be necessary for the correct assembly of a target protein structure. However, a chaperone protein cannot be a part of the resulting structure (Ellis, 1994). Because of its high hydrophobicity and high net negative charge at close to neutral pH, α_{s1}-, and β-casein could suppress the heat-induced aggregation of β-lactoglobulin solutions (O'Kennedy & Mounsey, 2006). These researchers showed a significant reduction in turbidity development in 0.5% (w/w) solutions of WPI when equal amounts of α_{s1}- and β-caseins were present in the system during heating at 85°C for 10 min. The addition of the combination of the two caseins did not affect the extent of denaturation but significantly reduced the formation of aggregates, which resulted in the reduced turbidity of the solution after heating, compared with the heated WPI solution. As both these caseins lack a free thiol group, it was suggested that the interactions were non-covalent in nature and probably reversible.

Spray drying and redispersion of the unheated powdered emulsion in water led to a very slight increase in the d_{43} values towards the larger size range. A total protein concentration of 3.0% (w/w) was observed in the previous study (Chapter 4) to be the optimum protein concentration required in emulsions, in addition to 12.0% (w/w) MD, to achieve good droplet stability during spray drying. Hence, this concentration was chosen to investigate the effect of heating on emulsions stabilized with whey proteins. The unheated emulsion containing 2.0% (w/w) WPI and 1.0% (w/w) NaCas also showed good stability upon drying and redispersion, as indicated by its size distribution (Fig. 7.2).

The stability of the oil droplets during spray drying of the emulsions was suggested in previous chapters as being dependent on (1) a saturated protein coverage on the oil droplet interface (with 20.0%, w/w, oil) and (2) a minimum unadsorbed protein concentration of ~ 1.0% (w/w) in the bulk phase of the emulsion (Taneja et al., 2013). During spray drying, a large air/liquid interface is created when the emulsion is atomised into a spray. Once this spray comes into contact with heated air inside the drier chamber, rapid evaporation of water from the spray takes place, turning it into powder particles (Elversson & Millqvist-Fureby, 2005). Along with water, protein molecules may also migrate, because of their high surface activity, towards the air/water interface (Kim et al., 2003).

It was suggested in previous chapters that, if sufficient unadsorbed protein molecules are present in the aqueous phase of the emulsion, the adsorbed protein molecules at the oil/water interface are under very little stress to desorb and migrate towards the air/water interface. However, if the emulsion contains insufficient unadsorbed monomeric protein in the aqueous phase, protein molecules may desorb

from the oil droplet interface and migrate to the air/water interface, resulting in the development of 'gaps' on the interface of the oil droplets, leading to oil droplet re-coalescence. Emulsions containing 3.0% (w/w) WPI were observed to have ~ 1.5% unadsorbed protein in the bulk phase, which resulted in the optimum stability of the oil droplets during spray drying. This was reflected by no noticeable change in the d_{43} values before and after spray drying. However, in emulsions made using < 2.0% (w/w) WPI, the unadsorbed protein concentration was < 1.0% and oil droplet re-coalescence was observed.

Heating of emulsions containing WPI only resulted in large-scale aggregation of the whey proteins in the aqueous phase and some aggregation on the surface of the oil droplets (Figs. 7.4 and 7.6). β-Lactoglobulin aggregates have also been shown to have lower surface activity than untreated native proteins (Millqvist-Fureby et al., 2001). This resultant aggregate formation and a decrease in monomeric whey proteins in the aqueous phase of the emulsions would have led to desorption of some of the protein molecules from the surface of the oil droplets during drying, leading to oil droplet re-coalescence. With the addition of NaCas, the aggregation on the surface of the oil droplets was lower in both sets of emulsions than in the untreated emulsions. It is very likely that the addition of NaCas would have prevented the aggregation of the whey proteins in the aqueous phase of the emulsions because of their tendency to act as chaperones, leaving the whey proteins in the monomeric state or as smaller aggregates. Therefore, with the majority of the whey proteins still in the monomeric state because of the addition of NaCas, upon heating, the desorption of the protein molecules adsorbed at the surface of the oil droplets would have been reduced, resulting in an improvement in stability during spray drying, compared with the heated WPI-only emulsions. There was an

improvement in the stability of the emulsions in both sets, i.e. NaCas added during the emulsification process or NaCas added post emulsification. However, the resulting average droplet diameter values were slightly lower for the emulsions in which NaCas was added during emulsification. This difference was probably due to the small differences in the concentration and the composition of the caseins in the aqueous phase and thus in the extent of aggregation of the whey proteins.

7.6 Conclusions

Milk proteins are usually found in combination in foods. NaCas is heat stable whereas whey proteins are sensitive to thermal treatments. Commercially available powdered foods have to be heat treated, usually at a high temperature for a short time, which could impact the redispersibility as well as other physicochemical properties of dried emulsions that consist mainly of whey protein. How can this sensitivity to thermal treatment be improved? In this work, we found that a noticeable improvement can be achieved with the addition of NaCas at one-third of the total protein concentration in whey-protein-stabilized emulsions. This effect occurred irrespective of whether the NaCas was added during homogenization or to the aqueous phase of the emulsions before heating. As long as casein proteins were present at the time of heating, the resulting spray-dried and redispersed emulsions had average droplet diameters in the same size range as their respective parent emulsions. The underlying mechanism of this stabilizing effect of NaCas in whey-protein-stabilized emulsions is related to the ability of casein proteins to provide steric stabilization to the emulsion droplets along with their ability to act as chaperones in the aqueous phase of the emulsions during heat treatment (Dickinson & Parkinson, 2004; Ellis, 1993; Guyomarc’h et al., 2009; O’Kennedy & Mounsey,

2006). Even though the impact of each individual casein protein on the aggregation kinetics of WPI solutions has been reported, the combined effect of caseins in the aqueous phase of whey-protein-stabilized emulsions has not been widely reported and needs further investigation.

Chapter 8

Emulsion composition and its impact on oxidative stability of the resulting spray dried powders

8.1 Abstract

Ten different emulsions, containing 20.0% (w/w) fish oil, 0.5-10.0% (w/w) protein (whey protein isolate and/or sodium caseinate) and 1.0-25.0% maltodextrin, were spray dried and investigated for oxidative stability over a storage period of 21 days as a function of their composition and application of heat treatment. A positive correlation ($P<0.05$) was seen between average droplet diameter (d_{43} values) of the redispersed emulsions and their respective extractable oil content. However, the emulsions which were prepared with preheat-treated protein solutions showed lower values of extractable oil content even though the average droplet diameter values of their redispersed emulsions were relatively larger. The PV and headspace propanal values correlated positively with each other ($p<0.05$). However, no correlation was seen between PV and headspace propanal values and extractable oil content. Also, a negative correlation was seen between the total protein content of the powders and the PV and headspace propanal values. Although, preheat treatment of the protein solution resulted in a shift in the d_{43} values of redispersed emulsions, the overall impact on oxidative stability of the resulting powders was positive.

8.2 Introduction

In the past two decades, within the functional foods and food supplements category, omega-3 (n-3) fatty acids, especially eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) obtained from marine sources, have gained much attention due to a vast list of associated health benefits (Drusch et al., 2007). The above-mentioned fatty acids fall under the long chain polyunsaturated fatty acid (LCPUFA) category and are considered highly desirable and safe by nutritionists worldwide for food fortification purposes (Drusch et al., 2007). Research into the heath benefits of n-3 LCPUFA has demonstrated positive health effects in the areas of neural development in infants, cardiovascular diseases (CVDs), platelet aggregation, hypertension, hyperlipidemia, cancer, dementia, Alzheimer's disease, depression and inflammation (Newton, 2001; Jacobsen et al., 2008; McManus et al., 2011).

The recommended intake of LCPUFA in daily diet for the prevention of heart disease is two serves of oily fish per week, which is equivalent to 200 mg of EPA and DHA per day. However, in several Western countries the average consumption is far below the recommended level (Kolanowski, 2006). The consumption of EPA and DHA can be increased either by increasing the intake of oily fish or by addition of fish oil in commonly consumed foods (Garg et al., 2006, Taneja & Singh, 2012). Increasing intake of fish has posed a challenge due to economic, environmental and cultural reasons. Furthermore, fortification of commonly consumed foods with fish oil is also challenging, as n-3 LCPUFAs are highly unsaturated in nature. This makes them increasingly susceptible to lipid oxidation in the presence of pro-oxidants such as atmospheric oxygen (Keogh et al., 2001). Oxidation renders the oil

organoleptically unacceptable to consumers (Hogan et al., 2003). This is the main limiting factor preventing the use of fish oil in functional foods (Chen et al., 2013). One way of preventing the n-3 LCPUFA from lipid oxidation, and making fortification less challenging, is by creating a barrier around the fish oil droplets which would prevent pro-oxidants from reacting with the fatty acids and forming oxidation by-products (Keogh et al., 2001; Hogan et al., 2003). This technique is generally referred to as microencapsulation (Keogh et al., 2001).

Microencapsulated powders containing oil are in fact homogenised emulsions where an emulsifying ingredient, such as milk proteins (whey protein, caseinates), soy proteins, modified starch (n-octenyl-succinate derivatized starch) or hydrocolloids (locust bean gum, gum arabic) have been used to produce sub-micron emulsion droplets. A filler material, such as a sugar (sucrose, lactose) or a hydrolysed starch product (maltodextrin), is also commonly used in the emulsions formulation to aid in the drying process (Drusch et al., 2007; Keogh et al., 2001). The added benefits of having fish oil in a powdered format is for allowing it to be added to powdered formulations and ease of blending in high moisture food formulations. Various methods have been previously proposed to convert fish oils, in the form of multi-oil blends and mixtures, into a multi-component powder. However, the most commonly used technique to remove moisture from emulsions is spray drying, due to the ease of availability of equipment and relatively low cost of manufacturing (Kolanowski et al., 2006).

In the previous chapters, the behaviour of oil droplets during spray drying of oil-in water emulsions stabilized with milk proteins was reported. The effects of spray drying on the redispersion behaviour as a function of the state and concentration of protein and maltodextrin concentration were explored. In addition, the impact of

pre-heat treatment of emulsions on the redispersion behaviour post spray drying was examined. The results showed that the composition of the parent emulsion played a significant role in stability of the emulsion droplets as it affected the droplet interface under the stressful environmental conditions during spray drying. Lipid oxidation in oil-in-water emulsions is also dependent on the nature of the droplet interface as the oxidation of fatty acids occurs at the interface (Katsuda et al., 2008). From this perspective, it is important to understand the correlation between the factors that impact the stability of powders containing fish oil i.e. type and concentration of milk proteins and maltodextrin, pre-heat treatment and oxidative stability.

8.3 Materials and methods

Whey protein isolate (WPI), sodium caseinate (NaCas) and maltodextrin (MD) were used in this study. The details of these materials and the equipment used in the study have been described in Chapter 3 (section 3.1 and 3.2). In addition, commercially available refined fish oil was used (DSM Nutritionals, Auckland, New Zealand). The fish oil contained approximately 35% total omega-3 fatty acids. Eicosapentanoic acids (EPA) and docosahexanoic acid (DHA) amounted to approximately 18% and 12% respectively. Fish oil also contained mixed tocopherols at 0.1% (w/w) which acted as antioxidants. A process flow diagram of the analyses conducted on the powdered emulsions is shown in Figure 8.1.

8.3.1 Emulsion preparation

A total of ten emulsion formulations were chosen for this study and have been presented in Table 8.1. The methods of emulsion preparation for these ten emulsions have been described in previous Chapter's methods sections (Chapter 4-

7). Emulsions E1 to E7 were prepared using the method described in section 4.3.1. Emulsions E8 and E10 were prepared using the method described in section 6.3.2. Emulsion E9 was prepared using the method described in section 7.3.1.

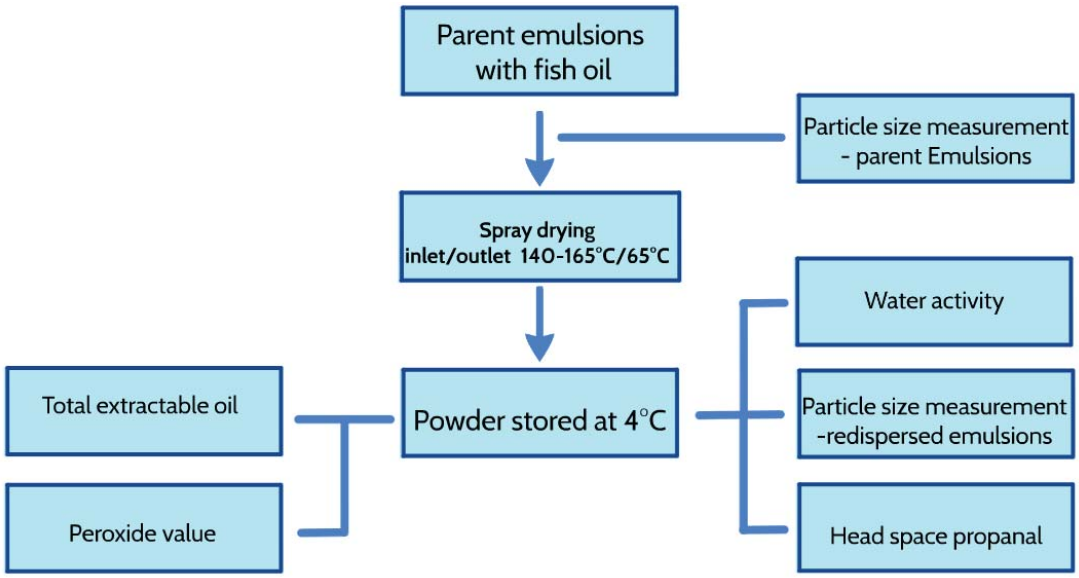


Figure 8.1 *Process flow diagram showing the analyses conducted on powdered emulsions for this study.*

8.3.2 Spray drying

All emulsions were spray dried using a Buchi bench-top spray drier (B-290, Buchi Labortechnik AG, Flawil, Switzerland) using standard glassware in co-current configuration and fitted with a two-fluid nozzle (nozzle cap 1.4 mm). The feed rate of the emulsion varied from 300 to 400 mL h^{-1}. The inlet temperature varied between 140 and 165 °C and the outlet temperature was maintained at 65 °C. The resulting powders were collected in air-tight containers.

Table 8.1 *Composition of experimental fish oil emulsions and powders*

Formulations	Emulsion (%, w/w)				Powder (%, d.b.)				PHT
	WPI	NaCas	Oil	MD	WPI	NaCas	Oil	MD	
E1	0.5	0	20.0	12	1.5	0	61.2	34.9	No
E2	3.0	0	20.0	12	8.2	0	56.9	32.4	No
E3	0.5	0	20.0	25	1.1	0	44.1	52.4	No
E4	10.0	0	20.0	1	30.6	0	63.9	3.0	No
E5	10.0	0	20.0	5	27.2	0	56.8	13.5	No
E6	0	0.5	20.0	12	0	1.5	61.2	34.9	No
E7	0	3.0	20.0	12	0	8.2	56.9	32.4	No
E8	3.0	0	20.0	12	1.5	0	61.2	34.9	Yes
E9	2.0	1.0	20.0	12	5.5	2.7	56.9	32.4	No
E10	2.0	1.0	20.0	12	5.5	2.7	56.9	32.4	Yes

Maltodextrin = MD; Pre-heat treatment = PHT (90°C for 10 min); Emulsion = E ; whey protien isolate = WPI; Sodium caseinate = NaCas

8.3.3 Determination of average droplet diameter

The diameters of the oil droplets for both the parent emulsions (fresh emulsions which have not been spray dried) and the redispersed emulsions (emulsions prepared by dissolving powder in RO water) were determined by the laser light scattering method as described by Jafari et al. (2007) and Ye (2011) using a Mastersizer E (Malvern Instruments, Malvern, Worcestershire, UK). Redispersed emulsions were prepared by reconstituting the dried emulsion powder in RO water to obtain the same total solids content as the parent emulsion. Powder was slowly added to RO water and the dispersions were stirred for at least 30 min before particle size measurements were made. In some cases, the emulsions were diluted 1:1 with 2.0% (w/w) sodium dodecyl sulphate (SDS) solution before the measurements were made. The comparison of the size distributions in RO water alone and with the addition of SDS indicated flocculation/aggregation during emulsification/redispersion (Sanchez & Patino, 2005). The droplet diameters were calculated as the averages of duplicate measurements.

8.3.4 Water activity

Water activity (a_w) of the spray-dried powders was measured using a water activity analyser (HydroLab 3, Rotronic, USA) at a temperature of 20°C approximately 24 hours after the powders were spray dried. Measurements were performed in duplicate.

8.3.5 Total extractable oil

The total extractable oil content of the powdered emulsions was determined using solvent (petroleum ether, boiling point 40–60 °C) extraction, as described by Kim et al. (2005). One gram of powder was carefully weighed on to a filter paper (No. 4

Whatman, Maidstone, Kent, UK) and was washed four times with 5 mL of petroleum ether. The mixture of solvent and oil was collected in a conical flask, evaporated over a steam bath and left overnight in the fume hood to allow further evaporation of the remaining ether. The flask was then placed in a 70 °C oven for 1 h and then cooled in a desiccator. The amount of extracted oil was recorded as milligrams of extractable oil per gram of powder.

8.3.6 Analysis of hydroperoxide content

For monitoring lipid oxidation, powders samples were stored at were stored 20°C after being spray dried for 4 hours in an opaque box to cool the powders down to room temperature. Following this the powder samples were placed in open jars at 20°C in a desiccator which contained a saturated solution of magnesium chloride providing a relative humidity of 33% (Drusch et al., 2007). Lipid hydroperoxide concentration was determined using a method adapted from Shantha and Decker (1994), Drusch and co-workers (2007) and Ries (2009). To 0.4 g of powder sample, 2 ml of an effective extraction solvent (ethanol/ethyl acetate/n-hexane, 1:1:1 ,v/v/v) was added and left at room temperature for 5 mins. Satue-Gracia et al (2000) reported the use of this solvent to successfully extract fat from infant formulas. This mixture was vortexed and later centrifuged to extract the lipids from the samples. Supernatant (0.2 ml) was transferred to a reaction tube with 3.8 ml of methanol/butanol (2:1; v/v). Thiocynate (30 µl) and ferrous iron solution (30 µl) was added. The sample tubes were vortexed for 2-4 s after each solution addition mentioned above. Control samples were prepared in the same way, except no powder was added. These tubes were kept in the dark for 5 mins before measuring the absorbance at 510 nm. A standard curve made with cumene hydroperoxide

diluted with the extraction solvent to various concentrations. These solutions were used instead of the sample extracts in the assay.

8.3.7 Determination of propanal by static headspace gas chromatography

Many studies have shown propanal as being a suitable marker for lipid oxidation, which is a byproduct from the degradation of omega-3 fatty acids (Serfert et al., 2009). To determine the propanal content in powders over a storage period of 21 days, static headspace gas chromatography method was used. Once the powder samples were allowed to cool for 4 hr in opaque box, 1g of powder samples was weighed in 20 mL crimp-sealed glass vials and was re-dissolved by adding 2 mL of EDTA solution (0.5%). Samples were equilibrated at 70 °C for 15 min. An aliquot of the headspace (1 mL) was injected into an Agilent 6890 gas chromatograph equipped with a HP-Innowax column (60 m Å~ 0.32 mm Å~ 0.5 μm) and an Agilent 5975 inert mass selective detector. The injector was operated in the split mode (5.3:1). Injector and detector temperatures were set at 270 and 250 °C, respectively.

Initially, the oven temperature was set at 50 °C and was held for 1.5 min. The temperature was increased to 240 °C at a rate of 20 °C min^{-1}, where it was held for 3 min. The mass spectrometer was operated in the electron ionization mode (70 eV), and data were acquired in the full-scan mode for the range m/z 20–200. The temperature of the ion source and the detector was 150 and 230 °C, respectively. Propanal was identified using the retention time of an external standard and by reference to the NIST library (NIST/EPA/NIH Mass Spectral Library, Version 2.0d, National Institute of Standards and Technology, Manchester, U.K.). The target ion

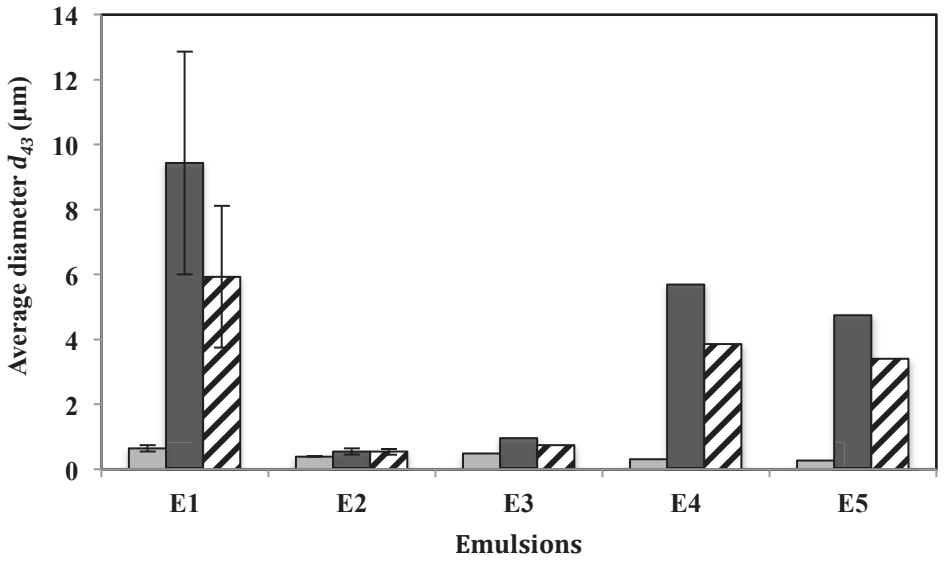
was 58; qualifiers were 29, 28, and 27. Quantification of propanal was done after calibration with known amounts of propanal standard.

8.3.8 Data analysis

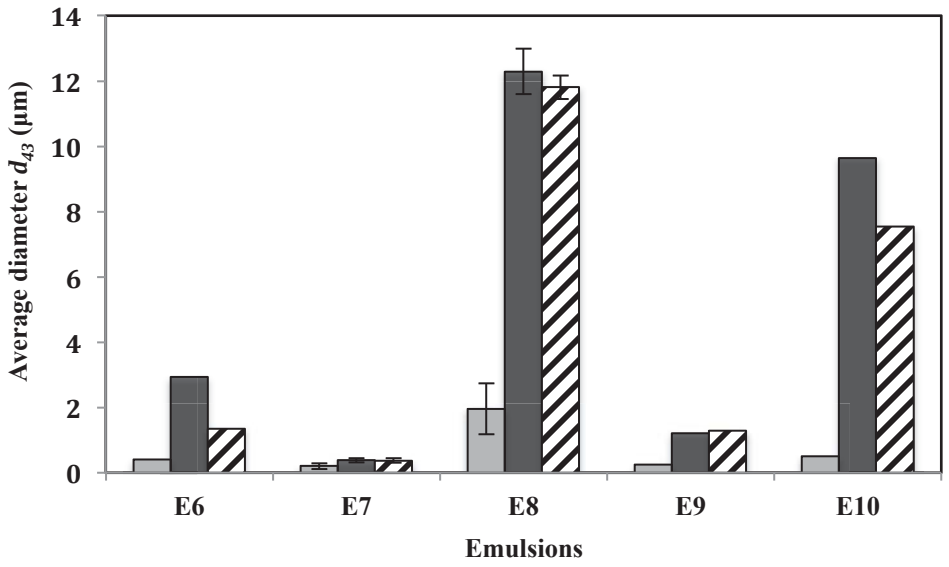
Each data point represents average value of two determinations on one emulsion. Error bars in graphs represent standard deviation of average value. Some graphs were drawn using Sigmaplot 13 graphing and data analysis software (Systat Software Inc., San Jose, CA, USA) and the other were drawn using Microsoft Excel 2011 (Microsoft Corporation, WA, USA).

8.4 Results

It is well known that in milk protein-stabilized emulsion systems, the composition of the emulsion affects the stability of oil droplets during spray drying (Drush et al., 2007; Hogan et al., 2001; Millqvist-Fureby et al., 2001; Singh and Ye, 2009). In the present study, different emulsion formulations (Table 8.1) were chosen from the previous experiments reported in Chapters 4, 6 and 7 (except E10) and soybean oil was replaced with fish oil. These ten emulsion formulation were chosen to represent emulsions which were unstable during drying and showed re-coalescence when redispersed after drying (E1, E4, E5, E6, E8 and E10) and the other emulsions which were stable during the drying process and resulted in powdered emulsions with average droplet diameter of the redispersed emulsions in similar size range as their corresponding parent emulsions (E2, E3, E7 and E9). The emulsions were investigated for changes in the oxidative stability, in relation to their respective compositions and behaviour during the spray drying process.



a)



b)

Figure 8.2 *Average droplet diameter (d_{43}) values for parent (grey bars) and redispersed (black bars) emulsions containing 20.0% (w/w) fish oil, 1.0-25.0% (w/w) maltodextrin and 0.5-10.0% (w/w) whey protein isolate and/or sodium caseinate. The redispersed emulsions were also diluted (1:1) with 2.0% (w/w) sodium dodecyl sulphate solution (striped bars).*

Figure 8.2 (a, b) shows the average droplet diameter values (d_{43}) of the parent emulsions (before drying) and upon redispersion in RO water after spray drying for various emulsions made using WPI and/or NaCas. All parent emulsions with a total protein concentration of ≥ 0.5% (w/w, WPI and/or NaCas) and ≥1.0%, (w/w) maltodextrin resulted in fine emulsions with narrow size distributions (Fig 8.2 a, b) with an average droplet diameter values of approximately 0.5 μm. The only exception was the parent emulsion made using pre-heat treated WPI solution at 90 °C for 10 mins which had an average droplet diameter value of approximately 2 μm (E8, Fig 8.2b). This was consistent with the results shown in Chapters 4, 6 and 7 (Fig 8.2 a, b). After spray drying, all powdered emulsions had a moisture content of 2.49 ± 0.51% (w/w) and an a_w value of 0.18 ± 0.02% (w/w). Powders below a moisture content of 5.0% are generally considered to be stable against microbial spoilage (Drusch et al., 2007).

The d_{43} of all redispersed emulsions shifted towards the larger size range as compared to their respective parent emulsion as reported earlier. Emulsions with either optimum concentration of protein (WPI and/or NaCas) and maltodextrin or optimum concentration of maltodextrin only (low protein) showed good redispersion behaviour i.e. the shift in the d_{43} values towards the larger values was only slight (E2, E3, E7, E9; Fig 8.2a, b). On the other hand, emulsions with lower concentrations of protein (<3.0%, w/w) and maltodextrin (<12.0%, w/w) showed larger shift in the d_{43} values towards the larger size range (E1, E4, E5, E6; Fig 8.2 a, b). Emulsion with low protein concentration but with higher MD concentration (>12.0%, w/w) showed only a slight increase in d_{43} values upon spray drying and redispersion (E3). However, in emulsions with lower concentration of MD (≤ 5.0%, w/w) but with higher concentration of proteins (≥3.0% w/w in emulsions), the d_{43}

values shifted noticeably towards the larger size values. Strikingly, the largest shift in the d_{43} values towards the larger size values within all emulsion variants was observed in the emulsion made using preheat-treated WPI solutions (E8; Fig 8.2 b). This shift in d_{43} values was mainly due to oil droplet re-coalescence, as observed by re-measuring the particle size after dilution of the redispersed emulsions in 2.0% (w/w) SDS solutions (Fig. 8.2 a, b).

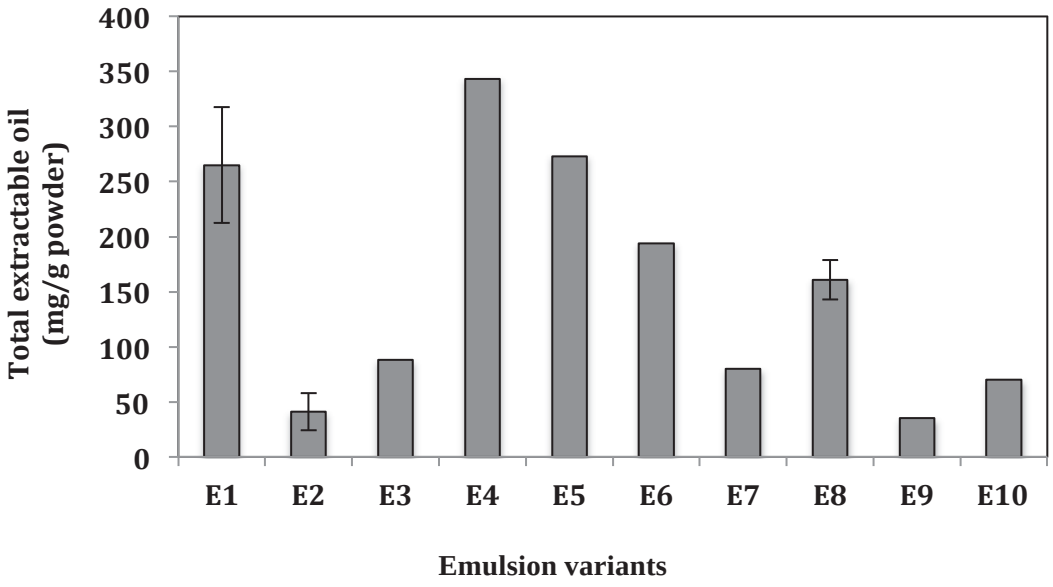


Figure 8.3 *Total extractable oil (mg/g powder) for powdered emulsions containing fish oil (42.9%-62.9% dry basis) with varying concentration of protein (whey protein isolate and/or sodium caseinate) and maltodextrin.*

Extractable oil (EO) values have been suggested as a measure of the oil holding capacity of powdered emulsions. Generally, lower values of extractable oil suggest that the oil is well preserved inside the powder matrix (Kolanowski et al., 2006,

Drusch et al., 2007). Emulsions E1 and E6 had similar protein, oil and MD concentration in the formulation. The main difference was the type of protein used for emulsions preparation i.e. WPI was used in E1 whereas NaCas was used to prepare E6. The OE value of E6 made using NaCas was observed to be slightly lower as compared to E1 made using WPI. This trend was similar to the change in d_{43} values before and after spray drying for these two emulsions (Fig 8.2). At relatively high protein concentrations, emulsion powders prepared using WPI were observed at ~50 mg g^{-1} which was slightly lower than the emulsion powders prepared using NaCas measured at ~80 mg g^{-1} at similar concentrations of protein.

Powder (E3) containing lower total protein (< 3.0%, w/w) but higher amount of maltodextrin (>12.0%, w/w) showed low value for extractable oil content. The extractable oil content was the highest (350 mg g^{-1}) in powder containing higher protein concentration but lower maltodextrin concentration (E4 and E5). These trends were similar to the trends seen in the change in d_{43} values towards the larger size range post spray drying and correlated positively ($p < 0.05$) (Table 8.2). Heat-treated emulsions were excluded from the Pearson correlation matrix, due to variables being non numeric in nature i.e. preheat treated/unheated.

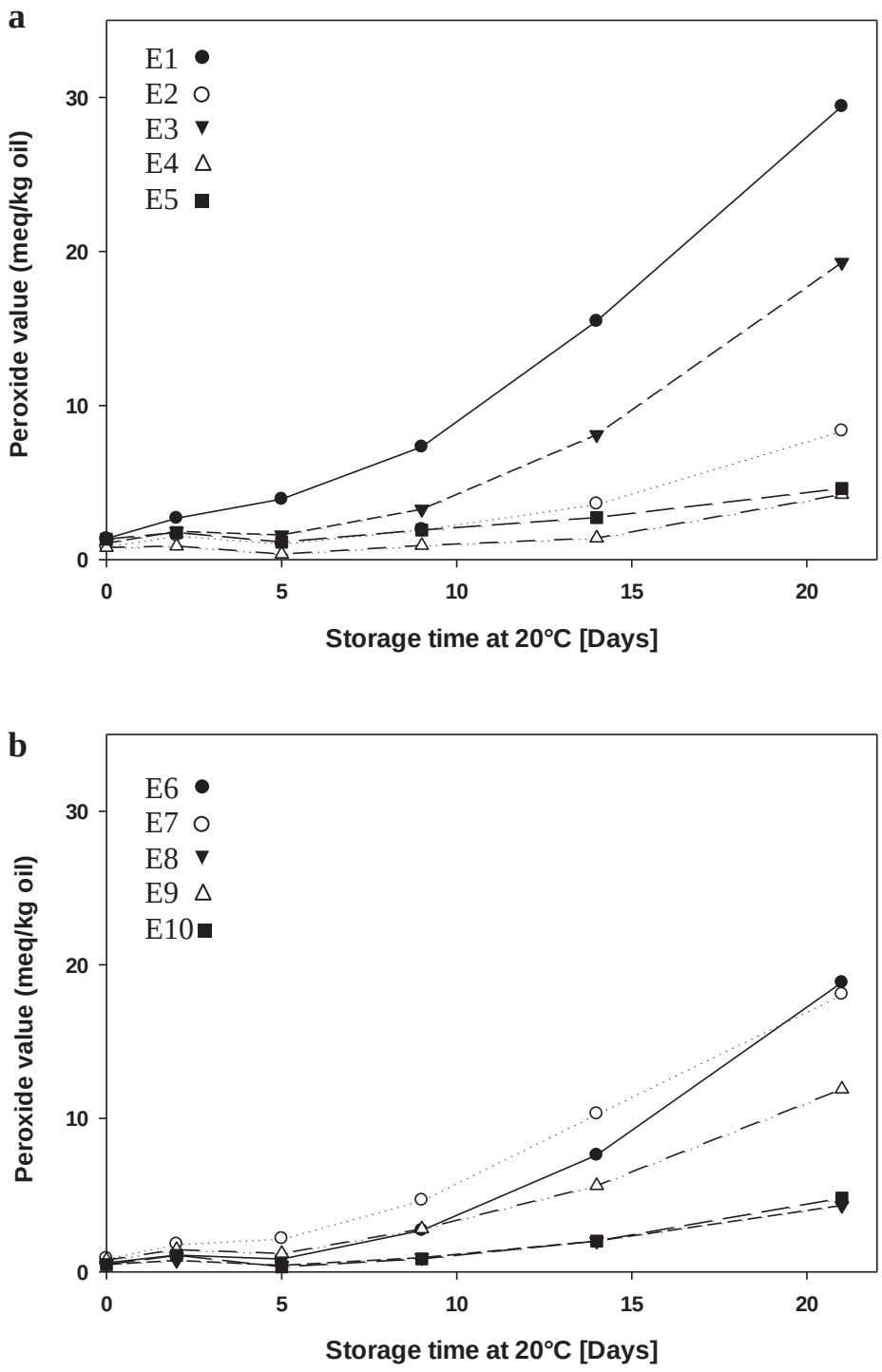


Figure 8.4 *Peroxide values (meq/kg oil) of various powdered emulsion formulations containing fish oil stored for 21 days at 20°C and 33% relative humidity.*

The preheat-treated emulsions were not included in the correlation matrix. The redispersed d_{43} values were the largest among the emulsion/powder variants for powders made using preheat-treated protein solutions (E8, E10; Fig. 8.2). However, their oil retaining capacity was much improved as compared with some of the other powder variants. Powdered emulsions made using preheat-treated WPI (E8) showed three times the extractable oil content as compared to unheated powdered emulsions containing the same concentration of WPI (E2). The impact of heat treatment on the d_{43} values of redispersed powdered emulsions (droplet stability during drying) in emulsion powder prepared using a mixture of preheat-treated proteins (E10). Even though, the protein content E10 was in the optimum range, due to heat treatment, the d_{43} values of E10 were in the same size range of the emulsion powders with low protein concentration (E1). However, the extractable oil content in E10 was five times less as compared with E1, indicating the positive impact of heat treatment on powder structure even though the particle size of redispersed emulsion revealed oil droplet re-coalescence during drying (Fig. 8.3).

Peroxide value (PV) is a standard food industry index to monitor quality, as it is an indicator of the initial stages of oxidative deterioration of oil (Wang et al., 2011). The changes in PV, which is a measure of amount of hydroperoxides present in oil in the spray dried powdered emulsions containing fish oil (in dry state), during 3 weeks storage at 20°C and 33.0% relative humidity are presented in Figure 8.4. The fastest increase in the PV was noticed in E1, which also showed a high content of extractable oil (Fig. 8.4 a). There was very little lag in the PV increase in this powder variant as the increase in the PV was observed from day 2 measurements. After a 3 week storage, the PV of this particular powder reached ~30 meq kg^{-1} oil. Emulsion powder E3, which also had a lower protein content but higher MD

concentration, also showed rapid increase in PV after a five day lag period reaching a PV of ~20 meq kg^{-1} oil after 3 weeks storage (Fig. 8.4a). A maximum PV value of 30 meq kg^{-1} oil has been recommended for edible food products (Gotoh & Wada, 2006; Wang et al., 2011). However, this maximum PV value is more applicable to foods containing vegetable oils. Moreover, human senses have very low threshold for detecting volatile off-flavours that are generated as a results of oxidation of fish oil. Jacobsen (2008) reported that a sensory panel was able to distinguish between fish oil-fortified milk samples with PV of 0.1meq/kg and 0.5 meq/kg. It was concluded in that study that in order for the fish oil-fortified milk samples to be acceptable for consumption; the PV should be <0.5 meq/kg (Jacobsen, 2008).

The other two powdered emulsions which also showed high PV at the end of the 3-week storage (~20 meq kg^{-1} oil) were E6 and E7 both of which were prepared using NaCas (Fig. 8.4 b). A higher PV for E7 was unexpected as this powdered emulsion showed a very slight shift in the d_{43} values towards larger size range upon spray drying and also showed low levels of EO content (Fig. 8.2- 8.3). Majority of the other powdered emulsions showed no change in the PV value till storage time of day 5-9. Interestingly, the powdered emulsions, which were pre-heat treated along with the high protein concentration, had the lowest PV by the end of the storage period, which remained below 5 meq kg^{-1} oil (Fig 8.4). When compared with unheated emulsion powders, heat-treated powder at the same composition had a lower PV. This was in contrast to the extractable oil content as well the redispersed emulsions d_{43} values (Fig 8.2-8.4). Protein concentration of emulsion powders showed a negative correlation ($p<0.05$) with PV as well (Table 8.2).

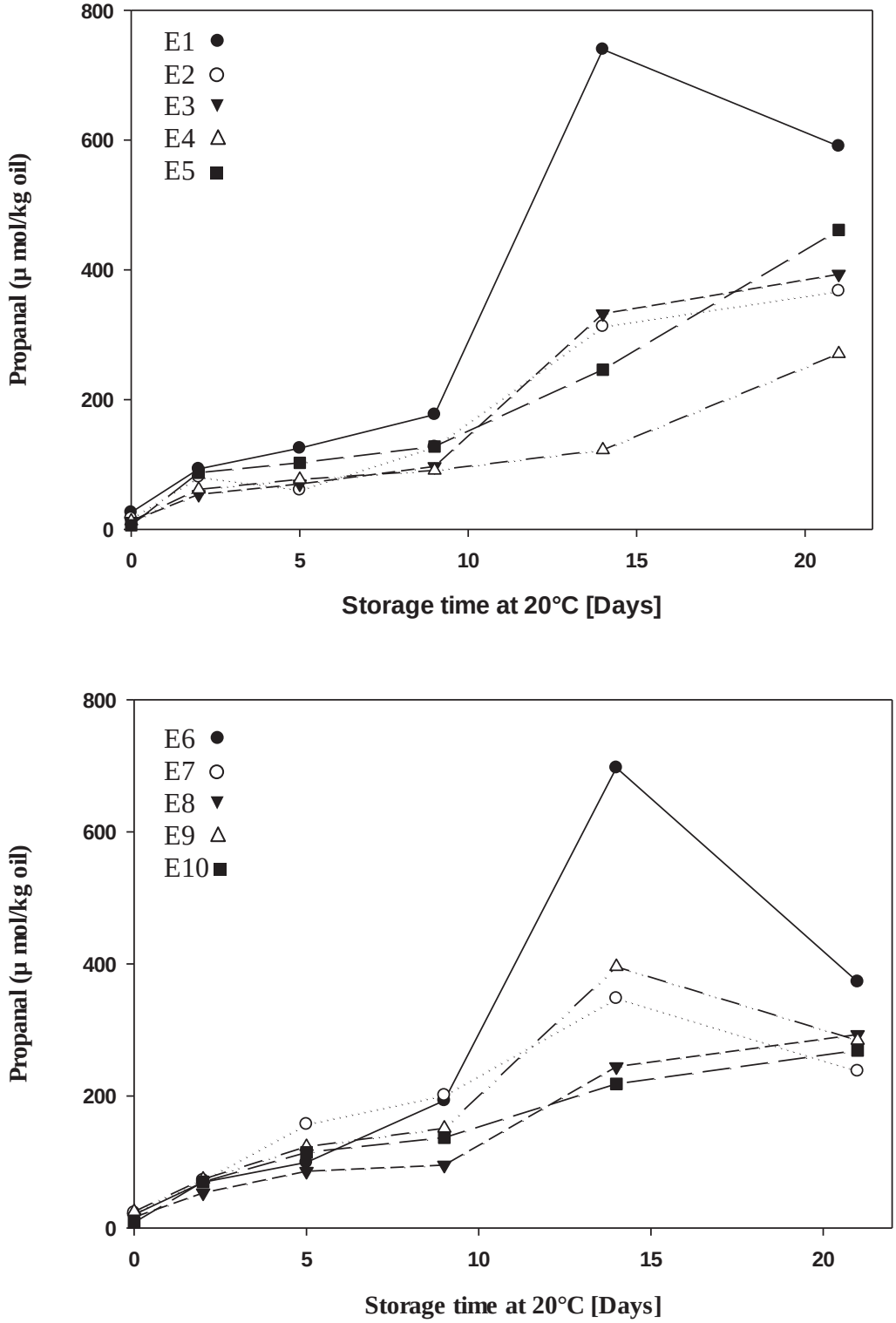


Figure 8.5 *Propanal content (µmol/kg oil) of various powdered emulsion formulations containing fish oil stored for 21 days at 20°C and 33% relative humidity.*

Some of the chemical compounds responsible for the characteristic sensory property of oxidized oils are aldehydes. Propanal is formed upon decomposition of lipid hydroperoxides via β-scission reaction (Katsuda et al., 2008). Therefore, headspace propanal has been used as an indicator of oxidation in oils, rich in n-3 fatty acids (Shen et al., 2007). The propanal values of these powdered emulsion showed a steady increase over the storage period (Fig. 8.5 a, b). A noticeable increase in the propanal content started at day 9 for nearly all the powder variants. The propanal content of emulsions containing lower concentration of protein showed a rapid increase (E1, E6). These powders also showed higher PV during storage. Emulsion powders with high protein content and low MD (E4, E5) also showed an increase in headspace propanal towards the end of shelf life but this increase was considerably lower than that in E1 and E6.

All other emulsion powders showed a steady increase in the propanal content similar to the trend in PV measurements and a positive correlation ($p<0.05$) was observed between propanal values at day 14 and PV at the end of storage. Although the d_{43} values of the redispersed emulsions showed a positive correlation with EO content, no significant correlation ($p>0.05$) was seen between d_{43} values of redispersed emulsions and PV and headspace propanal. However, as observed for peroxide values, the protein concentration of the emulsion powders correlated negatively ($p<0.05$) with headspace propanal values (Table 8.2).

Table 8.2 Pearson correlation (coefficient) matrix for various emulsion and powder (E1-E10) paramters.

	Protein Content (d.b.)	Oil Content (d.b.)	MD content (d.b.)	Parent emulsion d_{43} (µm)	Redispersed emulsion d_{43} (µm)	Extractable oil mg g^{-1}	PV Day 14 (meq kg^{-1} Oil)	PV Day 21(meq kg^{-1} Oil	Propanal Day 14 (µmol kg^{-1} oil)	Propanal Day 21 (µmol kg^{-1} oil)
Protein Content (d.b.)	1									
Oil Content (d.b.)	0.382662	1								
MD content (d.b.)	**-0.931744***	-0.692021	1							
Parent emulsion d_{43} (µm)	-0.540396	-0.096691	0.460775	1						
Redispersed emulsion d_{43} (µm)	0.316832	0.514727	-0.44948	0.475574	1					
Extractable oil mg g^{-1}	0.589025	0.563805	-0.681273	0.188055	**0.82032***	1				
PV Day 14 (meq kg^{-1} Oil)	**-0.729551***	-0.077369	0.60054	0.6072	0.247218	-0.094393	1			
PV Day 21 (meq kg^{-1} Oil)	**-0.819078***	-0.143197	0.69651	0.692183	0.181177	-0.118035	**0.972228***	1		
Propanal Day 14 (µmol kg^{-1} oil)	**-0.742352***	0.179524	0.50955	0.641867	0.237545	-0.013717	**0.777249***	**0.831825***	1	
Propanal Day 21 (µmol kg^{-1} oil)	-0.236537	-0.012612	0.189921	**0.795333***	0.657059	0.367441	0.476729	0.474354	0.554655	1

*p<0.05; E8 and E10 not included in correlation matrix.

8.5 Discussion

One of the most important factors that impacts emulsion stability during the drying process is the composition of the emulsion as well as the nature of the oil droplet interface. Furthermore, the physical and chemical properties of the resulting emulsion powders are partly affected by the stability of emulsion during drying (Drusch & Schwarz, 2006; Faldt & Bergenstahl, 1996). As reported in the previous chapters, emulsions stabilized with milk proteins (>2.0%, w/w) showed good stability against re-coalescence during drying, due to a saturated interfacial film on the surface of the oil droplet and a critical concentration of unadsorbed protein (~>1.0%, w/w) in the aqueous phase of the emulsion. These protein molecules in the aqueous phase of the emulsion possibly migrate to the air/water interface during drying and subsequently desorption of the protein molecules adsorbed at the oil/water interface. This stability is indicated by a small shift in the average droplet diameter values of these emulsions towards a larger size range before and after drying. This is the reason the excellent stability of emulsions containing optimum concentration of protein and maltodextrin (E2, E7 & E9, Fig. 8.2). It has also been reported that a critical amount of sugars or polyols is also required to effectively spray dry emulsions as they provide stability to the emulsion droplets under spray drying conditions (Faldt & Bergenstahl, 1996). This was seen in emulsions with high amount of proteins but low amount of maltodextrin (E4, E5) where a large shift in the droplet diameter upon spray drying and redispersion due to oil droplet re-coalescence was observed. However, a proportion of the protein present in the emulsion formulation can be substituted by maltodextrin (E3) to achieve good stability during spray drying.

Emulsions formed with pre-heat treated whey proteins (E8) showed a noticeable shift in the average droplet diameter values of the redispersed emulsions towards the larger size range as compared to emulsions made with unheated protein solution (E2). This was explained in Chapter 4 as probably due to protein denaturation and aggregation. The concentration of monomeric whey proteins in the aqueous phase of the emulsion before drying is crucial to the stability of emulsion droplets (Millqvist-Fureby et al., 2001; Dybowska, 2011). With the addition of NaCas to the whey protein solution during heating (1:2), the shift in the average droplet diameter of the redispersed emulsion was lower as compared with redispersed emulsions containing preheat treated WPI alone. As suggested in Chapter 5 NaCas may act as a chaperone preventing large-scale aggregation of the whey proteins in solution. (O'Kennedy & Mounsey, 2006). Therefore, with the larger proportion of the whey proteins still in the less-aggregated state, the resulting emulsions would show an improvement in stability during spray drying, compared with the heated WPI-only emulsions (E8, E10, Fig. 8.2).

According to Buma (1971) and Moreau & Rosenberg (1993), extractable oil reflects the degree to which the powder matrix can prevent extraction of the internal oil, through leaching with organic solvents. An inverse relationship has been previously reported between the parent emulsion particle size and the extractable oil content (Risch & Reineccius, 1988; Sheu & Rosenberg, 1995). A correlation between particle size of redispersed emulsions and the extractable oil of the resulting powders has not been conclusively reported in the literature (Drusch et al., 2007, Drusch & Berg, 2008; Soottitantawat et al., 2003, Hogan et al., 2001). In the present study, a positive correlation ($P<0.05$) was seen between average droplet diameter of the redispersed emulsions and their respective extractable oil content

(Table 8.2). This was indicative of poor oil retaining capability of emulsion powders, which either lacked an optimal concentration of protein and maltodextrin or higher concentration of maltodextrin to supplement the limited amount of proteins in emulsions during drying. With optimal emulsion formulation, the change in particle size was minimal upon spray drying which suggest that the resulting powder matrix had excellent barrier properties, possibly lower levels of oil on the surface of the powder particles and a dense compact structure which was impenetrable to oil extraction via organic solvent. In addition, higher protein content of the emulsion also provided oxidative stability regardless of the stability during drying.

Even though, the emulsion made using preheat treated protein solutions were not included in the Pearson correlation, emulsions E8 and E10 do not follow the same trend as compared to the other emulsions. Although, the average droplet diameter values of these redispersed emulsion showed a marked shift towards the larger size range before and after spray drying, the extractable oil values were noticeably lower than the other emulsion powder variants.

The amount of adsorbed whey protein on the interface of emulsion droplet has been reported to increase in emulsions heated at 90°C for 5 mins from 2.9 mg m^{-2} to 4 mg m^{-2} (Sliwinski et al., 2003; Singh & Ye, 2009). This would also be expected if emulsions were prepared using heat-treated whey proteins for emulsion powder E8 (Fig. 8.2-8.3). It would be expected that denatured/aggregated proteins would adsorb to the oil-water interface and the interface of the resulting emulsion would be thicker and denser. When a mixture of whey protein and caseins are used in preparation of emulsions, an increase in the total surface protein concentration has also been reported, as a result of closer packing of whey protein and caseins on the

droplet surface (Hunt & Dalgleish, 1994; Srinivasan et al, 1996; Ye, 2008). As not all of the extractable oil may be located on the surface (Buma, 1971); a thicker oil droplet interface as compared to low protein emulsion powder (E1, E6, possibly with a thinner interface) was possibly the reason for the lower extractable oil content observed.

The oxidative stability of fish oils has been reported extensively in literature. Even in the absence of air and light, fresh fish oil with low peroxide value (PV) and with added antioxidants, was shown to have an increase in the PV from 1 meq kg^{-1} to 4 meq kg^{-1} when stored at room temperature over a 30 day storage period. Spray drying process alone can accelerate the oxidation process of fish oil emulsions due to the presence of hot air inside the drying chamber. Studies have also shown that oxidation is inevitable once the fish oil emulsions have been exposed to elevated temperatures (Drusch et al., 2007; Kolanowski et al., 2006). Oxidation cannot be terminated, only slowed down, even with air-free packaging and dark storage conditions (Kolanowski et al., 2006). Extractable oil located on the surface of the powder particle has been suggested in the literature as being a key determinant of the shelf life of powder containing fish oils (Drusch et al., 2007). In this study, the PV and headspace propanal values correlated positively with each other ($p<0.05$). However, no correlation was seen between PV and headspace propanal values and extractable oil content. Interestingly, a negative correlation was seen between the total protein content of the powders and the PV and headspace propanal values. It has been reported that whey proteins can scavenge free radicals via by sulfhydryl groups and also chelate pro-oxidant metal ions, inhibiting lipid oxidations (Faraji et al., 2004). In addition, caseins showed metal binding capability due to a high content of phosphoseryl residues which in turn helps inhibit oxidation in emulsions

systems containing polyunsaturated fatty acids (Singh, 2011). Due to the oxidation inhibiting capability, the emulsion powders with lower concentration of protein were more susceptible (E1,E3, E6) to lipid oxidation as compared to emulsion powder with higher level of protein (E2, E4, E5, E7, E9; Fig. 8.3- 8.4). Even emulsion powders E4 and E5 which contained a high amount of protein but very low concentration of maltodextrin showed good storage stability.

Kiokias et al. (2007) reported a negative correlation between oxidative stability and the degree of whey protein denaturation. It was suggested that due to heat treatment of protein solution containing whey protein, a significantly higher proportion of reduced S-H groups are available which act as metal scavengers and thereby reduce the oxidation deterioration of emulsions containing fish oils (Sliwinski et al., 2003; Tong et al., 2000; Darka et al., 1998). This could possibly explain the increased stability of emulsion powders against lipid oxidation which were made using heat treated protein solutions as compared to the emulsion powders made with unheated protein solutions.

8.6 Conclusions

This study showed that the composition of the emulsion affects the stability of emulsion powder during drying. Stability during spray drying as indicated by average droplet diameter values on redispersion correlates well with the extractable oil content of powders. Emulsions showing a large shift in particle size before and after spray drying also showed higher extractable oil content. However, the relationship between extractable oil content and d_{43} values of redispersed emulsions was not straightforward. However, the oxidative deterioration of the emulsion powders could not be explained on the basis of extractable oil and d_{43} values of

redispersed emulsions alone. A negative correlation was also noticed between the total protein content of the emulsion powders and the oxidative stability measured through PV and headspace propanal content. Preheat treatment of the protein solution resulted in a shift in the d_{43} values of redispersed emulsions, the overall impact on oxidative stability of the resulting powders was positive.

Chapter 9

Overall discussion and avenues of future work

9.1 Overall discussion

Spray drying of emulsions has been used in the food industry for many decades (Vega & Roos, 2006). Most of the studies that have looked at emulsion stability have not extended the investigation further in dehydration of these emulsions into powders. In addition, the studies looking at powdered emulsion characterisation have primarily used common dairy powders, such as skim milk powder and whole milk powder. Only very few studies have used other model emulsions to investigate emulsion behaviour during spray drying. In most cases, the emulsions containing milk proteins and oil cannot be dried as is. Incorporation of low molecular weight sugars is important to stabilize these emulsions during drying. The concentration of these sugars also has a significant impact on the stability of the resulting powder along with the main stabilizers in these formulations i.e. milk proteins. This study is the first systematic attempt looking at the behaviour of emulsions during spray drying of milk protein-stabilized emulsions as a function of its composition (including the concentration of sugars) and processing conditions (pre-heat treatment) applied to the emulsion before drying.

Among the most important properties of dehydrated emulsions, redispersion behaviour and storage stability stand out noticeably. Therefore in this study, the stability of the emulsions was investigated by observing primarily the changes in particle size of emulsions before and after drying as it is one of the most important properties of powdered emulsions from a commercial stand point (Vega & Roos,

2006). Storage stability was also investigated at later stage in this study. Spray drying of emulsions with low protein concentration (<2.0%, w/w) resulted in a shift in the average droplet diameter upon redispersion in emulsions made using WPI or NaCas. This shift was thought to be due to oil droplet coalescence, which was confirmed by diluting (1:1) the emulsions with SDS solution (2.0%, w/w) before particle size measurements were made.

Coalescence was confirmed in these emulsions using confocal laser scanning microscopy (CLSM) as coalesced oil droplets were observed in the intact powder particles made using low protein concentration (<2.0%, w/w) as compared with powdered emulsions containing high protein concentration (>2.0%, w/w) where smaller sized oil droplet were well dispersed within the powder matrix (Chapter 4). CLSM of powder particle was quite challenging for emulsions prepared in this research as even a small amount of water in the medium used to fix powders on the microscopy slides would lead to in poor image quality and a change in the morphology of the powder particles while imaging. Therefore, the dyes were included in the oil and aqueous phase of the emulsion before spray drying during our experiments, which resulted in powders already containing these fluorescent dyes. In addition DPX, which is non-aqueous mounting medium, was used which resulted in excellent resolution of confocal micrographs clearly showing a difference in the oil droplet size for high and low protein content powders (Chapter 4, 5).

The role of sugars (in this study maltodextrin) was also found to be crucial as even at very high concentration of protein (~10%, w/w) in the emulsions, the absence of sugars led to coalescence of oil droplet in the resulting powder as the particle

showed a marked shift towards the larger values. This was suggested to be due to the ability of sugars to form a viscous glass upon dehydration, which possibly prevented any major conformational changes and/or mobility of the proteins from the oil droplet interface. At an optimum concentration of protein and maltodextrin, the size distribution of the parent emulsions (before drying) overlapped with the distribution of redispersed emulsions.

Coalescence of oil droplet during drying at low protein concentration is not well understood. Even though 1.0% (w/w) protein in the aqueous phase was enough to create a stable emulsion, the stability during drying was not maintained. It was suggested in this study that the possible reason for coalescence of the oil droplet in emulsions containing <2.0% (w/w) could be due to the migration of the adsorbed protein molecules on the surface of the oil droplets to the newly created air/water interface of the spray droplet as a result of atomisation. Emulsions not only must have oil droplets saturated with proteins at the interface, a sufficient amount of protein molecules must also be present in the bulk phase of these emulsions to fully cover this newly created air/water interface. If insufficient protein exists in the bulk phase of the emulsion, migration of protein from the surface of the oil droplet to the air/water interface could occur creating “gaps” in the interface, which may lead to coalescence of the oil droplets. As expected, this effect would be less at higher concentrations of protein in the continuous phase and hence the overlapping size distributions before and after drying. The amount of unadsorbed protein in the bulk phase were measured in this study and it was suggested that a $\geq$ 1.0% (w/w) unadsorbed protein in the aqueous phase of the emulsion would provide good stability during drying (Fig. 9.1).

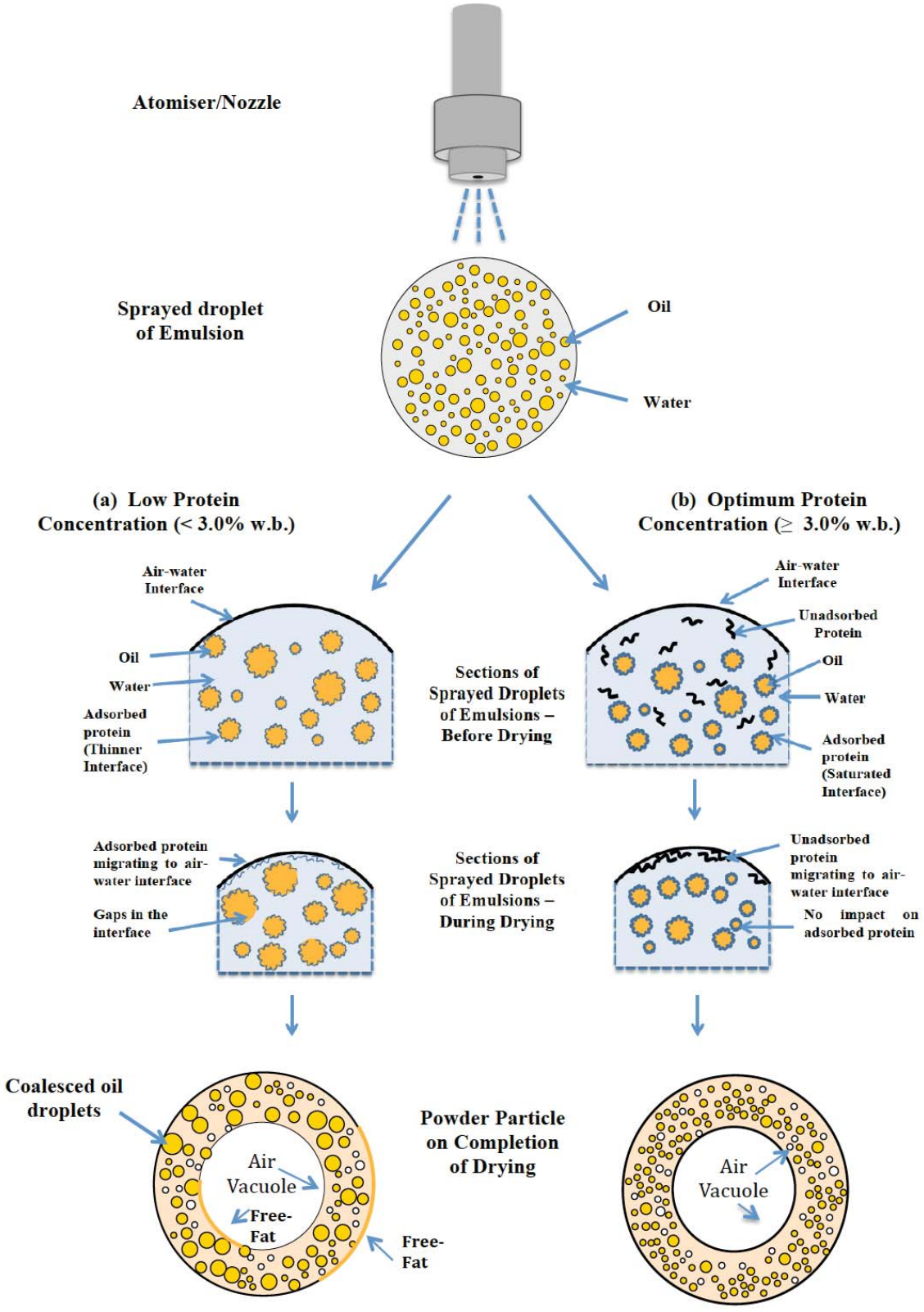


Figure 9.1 *Suggested course during spray drying of sprayed emulsion droplets, when (a) protein is present at a low concentration in the emulsion and (b) protein concentration is optimum.*

The type of protein molecules also seemed to have an impact on the stability during drying. WPI and NaCas mainly consist of individual (monomeric) protein molecules and only a small quantity of aggregate proteins. On the other hand, 25% of the total protein present in CaCas has been reported as being aggregated (Srinivasan et al., 1999). Similarly, MPC contains caseins, which remain in native form and therefore have a large proportion of caseins present as aggregates (Singh & Ye, 2009). As larger aggregated molecules are less surface active, the concentration of monomeric protein species was suggested to be crucial for stability during drying. Therefore, for emulsions prepared with MPC or CaCas, the optimum concentration at which the shift in droplet diameter was minimal was higher as compared to emulsions made using WPI or NaCas.

During commercial processing, emulsions are heated before being spray dried, as regulatory authorities legally require this. Depending on the microbial quality of the raw materials, often high heat treatment >80°C is applied. Heat treatment can be applied at the raw material supplier's facility i.e. to the raw material itself or during processing of the finished product i.e. emulsion processing and drying. Both scenarios were investigated in chapter 6 for emulsions prepared using WPI only.

Emulsions made using pre-heat treated WPI solutions (65-90 °C for 10 mins) showed a shift in their average droplet diameters towards the larger size range once the emulsions were prepared. In addition, upon spray drying and redispersion, further shift in the droplet diameter towards larger values was observed. This shift was supported by the hypothesis presented in Chapter 4 as the heat-treated emulsions would have a lower concentration of monomeric protein in the bulk phase of the emulsion as compared to unheated emulsions. This could result in

migration of the monomeric protein from the bulk phase of the emulsion towards the air/water interface (Chapter 6).

When emulsions were heat-treated instead of the protein solutions, no change in size distribution was observed in the emulsions upon heat treatment. However, a shift in the droplet diameter towards the larger size range was seen once these heat-treated emulsions were spray dried. The results of SDS-PAGE and droplet size suggested that there may be an effect of the size of aggregated that are formed upon heat treatment as well. At high concentration of protein, large sized aggregated formed would either be present on the interface of the oil droplets or in the powder matrix. These large sized aggregates could potentially rupture the stabilizing film on the oil droplet interface leading to coalescence either during the powder manufacture or during the redispersion of powder in water.

Recent studies have shown that the addition of caseins can protect whey proteins from the adverse effects of heat treatment even at a very low concentration (Dickinson & Parkinson, 2004). Therefore, the findings from Chapter 6 were extended by using a mixture of WPI and NaCas for preparation of emulsions to observe the impact of heat treatment on stability of emulsions during drying. When mixtures of WPI and NaCas were used to prepare emulsion, both proteins were present on the surface of the emulsion droplet as seen in SDS-PAGE results of the cream phase. Even when the NaCas solution was added to a preformed emulsion (after emulsification), both proteins were still visibly evident (Chapter 7). In both cases i.e. NaCas added before emulsification and after emulsification, the shift in the droplet size towards larger size range was reduced. This further solidified the hypothesis presented earlier that if aggregation is retarded, higher amount of

monomeric or less aggregated protein in the bulk phase of emulsions would stabilize the emulsion droplet during drying.

Lastly, the finding from previous chapter was linked with another important property of dried emulsions i.e. storage stability. The impact of composition and processing on the oxidative stability was investigated in the last section of this study. A positive correlation was observed between the average droplet diameter of the redispersed emulsion and the extractable oil of the powder. This was indicative of poor oil retaining capability of powders, which either lacked optimal concentration of protein and maltodextrin or higher concentration of maltodextrin to supplement the lack of presence of sufficient proteins in emulsions during drying. With optimal emulsion formulation, the change in particle size is minimal upon spray drying which suggest that the resulting powder matrix had excellent barrier properties, possibly lower levels of oil on the surface of the powder particles and a dense compact structure which was impenetrable to oil extraction via organic solvent. When the change in particle size In addition, higher protein content of the emulsion also provided oxidative stability regardless of the stability during drying. However, as reported in other studies, high extractable oil content does not necessarily mean poor oxidative stability. In conclusion, stability of emulsions during drying and oxidative stability of powders are not governed by the same factors.

Overall, the study has added a number of useful new insights about the mechanism behind oil droplet coalescence during the drying of emulsions. It is indeed possible to have minimal change in the emulsion properties if optimum concentration of its components are used even when the total oil content of the powder is >50% (d.b.). This preservation of the oil droplet size also reflects on the oxidative stability of

such powders. Preheating of either whey protein solutions or heating of the emulsion containing whey proteins results in marked change in the droplet diameter but the oxidative stability of these powders is still maintained or impaired.

9.2 Avenues of future work

In light of outcomes from this study, potential areas for further research are recommended below.

- The powders produced in this research were dried using a bench top spray dryer with two fluid nozzle. Two fluid nozzles are not widely used in industrial spray drying plants. Also, the control over drying conditions and powder parameters cannot be entirely controlled in a bench top spray drier. Thus, a detailed investigation, similar to that done in Chapters 4 and 5, may be carried out using a pilot-scale spray drier fitted with pressure nozzles, internal fluid beds and ability to return fine particles for better control over bulk density. This could provide better understanding of emulsion stability in a drier set up which resembles industrial drying operations.
- The breakdown on emulsion droplets during atomisation was explained briefly in Chapter 4, but this topic has received little attention in the literature. More work may be carried to understand the intensity of breakdown during atomisation as a function of in-feed total solids and amount of air used for atomising the emulsion.
- The total solids of the in-feed or wet phase used in this study was 30-37% (w/w). Commercially, much higher total solids are used during drying, as it is not economically viable to add excess water that is later removed from the formulation. A total solids of 50-60% (w/w) is commonly used in

industry that is obtained by falling film evaporation before drying. Work represented in Chapters 4 and 5 could be extended at higher total solids in order to study the impact of higher total solids on emulsion stability during drying.

- The preheating of protein solutions and the heat treatment of emulsions was done using a water bath, which took some time for the temperature to reach desired value. Pilot equipment that is able to reach high temperature faster such as direct steam injection couple with a flashing vessel to cool emulsions/solutions down may be used to investigate the effects of heating further.
- Heating of emulsion was only conducted up till 90°C, whereas commercially applicable heat treatment such as ultra high heat treatment (UHT) e.g. 121°C for 10 sec are higher temperature but for a short time. UHT may be used to investigate the impact on whey protein aggregation and its impact on emulsion stability in the model emulsion systems used in these experiments.
- Reconstitution behaviour of a powdered emulsion is one of the most critical properties from a consumer acceptability perspective. It is common to have recommendations of a short reconstitution time on the product labels of powdered finished products (whole milk powder, infant formula, toddler formula) for consumers convenience. In these experiments, the reconstitution behaviour of the powdered emulsions was looked at from a droplet size viewpoint. It is suggested that reconstitution behaviour of the powdered emulsions may also be looked at from a solution appearance point of view (i.e. by pouring reconstituted emulsion over a glass slab) to observe

whether the particles have fully dissolved as a function of reconstitution time as undissolved particles (white flecks) or sedimentation is commonly observed in milk protein containing formulations upon reconstitution of finished products as per the recommendations/directions on the pack label.

- In most microencapsulation systems two types of constituents exist; i.e. a film forming or emulsifying component and a bulk or a filler component. Key parameters that impact the oxidative stability of n-3 PUFA in such systems is the presence of free fat present on the surface of the particle and the amount of oxygen available in the emulsion and in the finished powder for oxidation. Previous patents (Singh, Zhu & Ye, 2006) have exploited chemical interactions between caseinates and whey proteins to improve the encapsulated fish oil stability in liquid emulsion systems, however further research is warranted to extend this understanding in powdered systems and to understand the correlation between processing conditions (heating time/temperature etc.) and key stability factors (available oxygen and surface free fat). This knowledge could be useful for the development of robust milk protein based encapsulation systems that aid in the incorporation of n-3 PUFA in food products.
- Several studies have explored the mechanisms by which small molecular weight surfactants (SMS) displace milk proteins at oil-water and air-water interface. In emulsions, the combination of SMS and proteins has huge implications on foaming and emulsion stabilizing properties. Commonly used SMS are mono and diglycerides, phospholipids, polysorbates, lecithin etc. The mechanisms by which proteins and SMS stabilize emulsions are very different. Proteins stabilize emulsions via the formation of an immobile

viscoelastic network whereas SMS, due to their high degree of mobility, stabilize emulsions by Gibbs-Marangoni mechanism. Often in commercial products, both proteins and SMS are present. Further understanding is required to better understand the impact of addition of SMS in milk-protein stabilized emulsions on the stability of the oil droplets during drying and the susceptibility of such powders to oxidation.

- Food powders are generally characterised using bulk parameters and scientific descriptions of such powders still need further development. New experimental techniques are continually being developed which enable characterisation of powder surfaces in order to better understand the impact of surface characterisation on bulk functional properties. It is suggested that these techniques should be explored further to characterise high fat milk protein based powders in order to better understand the mechanisms responsible for the stabilisation of emulsion droplets during drying
    - Electron spectroscopy of chemical analysis (ESCA): ESCA is capable of providing elemental data of the powder surface up to a depth of 10 nm. This technique has been used in recent studies to determine the surface protein, fat and lactose composition.
    - Atomic force microscopy (AFM): AFM can provide information relating to the surface topography and has been used to study the structure of biopolymers such as pectin, xanthan and various botanical sources of starch granules (e.g. wheat, pea, rice etc.).
    - Laser light scattering and laser diffraction: the wetting behaviour of powders depends upon particle morphology, which can be studied using laser diffraction techniques. Circularity as well elongation of

food powders can be determined using laser diffraction system coupled with image analysis tool.

References

Adhikari, B., Howes, T., Bhandari, B. R., & Langrish, T. A. G. (2009). Effect of addition of proteins on the production of amorphous sucrose powder through spray drying. *Journal of Food Engineering, 94*, 144−153.

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.

Aguilera, J., del Valle, J., & Karel, M. (1995). Caking phenomena in amorphous food powders. *Trends in Food Science & Technology*, *6*, 149-155.

Anema, S. G., & Li, Y. (2003). Association of denatured whey proteins with casein micelles in heated reconstituted skim milk and its effect on casein micelle size. *Journal of Dairy Research, 70*, 73-83.

Appelqvist, I. A., Golding, M., Vreeker, R., & Zuidam, N. J. (2007). Emulsions as delivery systems in foods. *Encapsulation and controlled release technologies in food systems*, 41-81.

Auty, M. A. E., Twomey, M., Guinee, T. P., & Mulvihill, D. M. (2001). Development and application of confocal scanning laser microscopy methods for studying the distribution of fat and protein in selected dairy products. *Journal of Dairy Research, 68*, 417−427.

Barrier, P., & Rousseau, J.Y. (1998). Powder comprising microcapsules based on fish oils rich polyunsaturated fatty acids, *French patent application*, FR2758055.

Barrow, C., & Shahidi, F. (Eds.). (2007). *Marine nutraceuticals and functional foods*. CRC Press.

Bhandari, B. R., & Howes, T. (1999). Implication of glass transition for the drying and stability of dried foods. *Journal of Food Engineering, 40*, 71-79.

Birchal, V. S., Huang, L., Mujumdar, A. S., & Passos, M. L. (2006). Spray dryers: modeling and simulation. *Drying Technology*, *24*, 359-371.

Blijdenstein, T. B. J., van Vliet, T., van der Linden, E., & Van Aken, G. A. (2003). Suppression of depletion flocculation in oil-in-water emulsions: a kinetic effect of β-lactoglobulin. *Food Hydrocolloids*, *17*, 661-669.

Blijdenstein, T. B. J., Van Winden, A. J. M., Van Vliet, T., Van der Linden, E., & Van Aken, G. A. (2004a). Serum separation and structure of depletion-and bridging-flocculated emulsions: a comparison. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *245*, 41-48.

Blijdenstein, T. B. J., Van Winden, A. J. M., Van Vliet, T., Van der Linden, E., & Van Aken, G. A. (2004b). Serum separation and structure of depletion-and bridging-flocculated emulsions: a comparison. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *245*, 41-48.

Brew, K., & Grobler, J. A., (1992). α-Lactalbumin. In P. F. Fox (Ed.), *Advanced dairy chemistry-1: Proteins.*, (Ed. 2), 191-229. New York, US: Elsevier Inc.

Bronlund, J., & Paterson, T. (2004). Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *International Dairy Journal*, *14*, 247-254.

Buchheim, W. (1982). Electron microscopic localization of solvent-extractable fat in agglomerated spray-dried whole milk powder particles. *Journal of Food Microstructure*, *1*, 12.

Buma, T. J. (1971a). Free fat and physical structure of spray-dried whole milk. Doctor of Natural Science Thesis, Landbouwhogeschool, Wageningen, The Netherlands.

Buma, T. J. (1971b). Free fat in spray-dried whole milk. 10. A final report with a physical model for free fat in spray-dried milk. *Nederlands melk-en zuiveltijdschrift*.

Chang, L. Q., & Pikal, M. J. (2009). Mechanisms of protein stabilization in the solid state. *Journal of Pharmaceutical Sciences, 98*, 2886−2908.

Chen, Q., McGillivray, D., Wen, J., Zhong, F., Quek, S. Y. (2013). Co-encapsulation of fish oil with phytosterol esters and limonene by milk proteins. Journal of Food Engineering, *117*, 505-512.

Christensen, K. L., Pedersen, G. P., & Kristensen, H. G. (2002). Physical stability of redispersible dry emulsions containing amorphous sucrose. *European journal of Pharmaceutics and Biopharmaceutics*, *53*, 147-153.

Chronakis, I. S. (1998). On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: a review. *Critical Reviews in Food Science and Nutrition*, *38*, 599-637.

Cicerone, M. T., & Douglas, J. F. (2012). β-Relaxation governs protein stability in sugar-glass matrices. *Soft Matter, 8*, 2983−2991.

Dalgleish, D. G. (1993). The sizes and conformations of the proteins in adsorbed layers of individual caseins on latices and in oil-in-water emulsions. *Colloids and Surfaces B: Biointerfaces, 1*, 1-8.

Dalgleish, D. G. (1996). Conformations and structures of milk proteins adsorbed to oil-water interfaces. *Food Research International*, *29*, 541-547.

Dalgleish, D. G., Goff, H. D., Brun, J. M., & Luan, B. B. (2002). Exchange reactions between whey proteins and caseins in heated soya oil-in-water emulsion systems–overall aspects of the reaction. *Food Hydrocolloids*, 16, 303–311.

Dalgleish, D. G., Senaratne, V., & Francois, S. (1997). Interactions between α-lactalbumin and β-lactoglobulin in the early stages of heat denaturation. *Journal of Agricultural and Food Chemistry*, *45*, 3459-3464.

Damodaran, S., & Agyare, K. K. (2013). Effect of thermal transglutaminase treatment on thermal stability and pH-solubility of heat shocked whey protein isolate. *Food Hydrocolloids*, 30, 12-18.

Damodaran, S., & Anand, K. (1997). Sulfhydryl-disulfide interchange-induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. *Journal of Agricultural and Food Chemistry*, *45*, 3813-3820.

Damodaran, S., & Kamlesh A. (1997). Sulfhydryl-disulfide interchange-induced interparticle protein polymerization in whey protein-stabilized emulsions and its relation to emulsion stability. *Journal of Agricultural and Food Chemistry*, 45, 3813-3820.

Dannenberg, F., & Kessler, H. G. (1988). Reaction kinetics on the denaturation of whey proteins in milk. *Journal of Food Science*, 53, 258–263.

Darka, J., Mundoma, C., & Simoyi, R. H. (1998). Antioxidant chemistry: Reactivity and oxidation of dl-cysteine by some common oxidants. *Journal of the Chemical Society, Faraday Transactions*, 94, 1971–1978.

Darling, D. F., & Birkett, R. J., (1987). Food colloids in practice. In E. Dickinson (Ed.), *Food emulsions and foams (pp. 1-29)*. London, UK: Woodhead Publishings.

De Hek, H., & Vrij, A. (1981). Interactions in mixtures of colloidal silica spheres and polystyrene molecules in cyclohexane: I. Phase separations. *Journal of Colloid and Interface Science*, 84, 409-422.

de la Fuente, M. A., Singh, H., & Hemar, Y. (2002). Recent advances in the characterisation of heat-induced aggregates and intermediates of whey proteins. *Trends in Food Science & Technology*, 13, 262–274.

del Angel, C. R., & Dalgleish, D. G. (2006). Structures and some properties of soluble protein complexes formed by the heating of reconstituted skim milk powder. *Food Research International*, *39*, 472-479.

Demetriades, K., Coupland, J. N., & McClements, D. J. (1997). Physicochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. *Journal of Food Science*, 62, 462–467.

Dickinson, E. (1999). Caseins in emulsions: interfacial properties and interactions. *International Dairy Journal*, 9, 305-312.

Dickinson, E. (1992). Introduction to food colloids. London, UK: *Oxford University Press*.

Dickinson, E. (1996). Biopolymer interactions in emulsion systems: Influences on creaming, flocculation and rheology. *ACS Symposium*, 650, 197-207.

Dickinson, E. (1998). Proteins at interfaces and in emulsions stability, rheology and interactions. *Journal of the Chemical Society, Faraday Transactions*, *94*, 1657-1669.

Dickinson, E. (1999). Caseins in emulsions: interfacial properties and interactions. *International Dairy Journal*, *9*, 305-312.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*, 25-39.

Dickinson, E. (2008). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, *23*, 1473–1482.

Dickinson, E., & Euston, S. R. (1991). Stability of food emulsions containing both protein and polysaccharide. In E. Dickinson (Ed.), *Food emulsions, gels and colloids* (pp. 132-146). Cambridge, UK: Royal Society of Chemistry.

Dickinson, E., & Golding, M. (1997). Depletion flocculation of emulsions containing unadsorbed sodium caseinate. *Food Hydrocolloids*, *11*, 13-18.

Dickinson, E., & Matsumura, Y. (1991). Time-dependent polymerization of β-lactoglobulin through disulphide bonds at the oil-water interface in emulsions. *International Journal of Biological Macromolecules*, *13*, 26-30.

Dickinson, E., & McClements, D. J. (1995). Advances in food colloids. *Springer Science & Business Media.*

Dickinson, E., & Parkinson, E. L. (2004). Heat-induced aggregation of milk protein stabilized emulsions: sensitivity to processing and composition. *International Dairy Journal, 14,* 635–645.

Dickinson, E., & Pawlowsky, K. (1997). Effect of ι-carrageenan on flocculation, creaming, and rheology of a protein-stabilized emulsion. *Journal of Agricultural and Food Chemistry, 45,* 3799-3806.

Dickinson, E., & Stainsby, G. (1982). *Colloids in food*. Applied Science Publishers.

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *Journal of Agricultural and Food Chemistry, 44,* 1371-1377.

Dickinson, E., Murray, B. S., & Stainsby, G. (1988). Coalescence stability of emulsion-sized droplets at a planar oil–water interface and the relationship to protein film surface rheology. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases, 84,* 871-883.

Dollo, G., Le Corre, P., Guérin, A., Chevanne, F., Burgot, J. L., & Leverge, R. (2003). Spray-dried redispersible oil-in-water emulsion to improve oral bioavailability of poorly soluble drugs. *European Journal of Pharmaceutical Sciences, 19,* 273-280.

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.

Drusch, S., & Mannino, S. (2009). Patent-based review on industrial approaches for the microencapsulation of oils rich in polyunsaturated fatty acids. *Trends in Food Science and Technology*, *20*, 237-244.

Drusch, S., & Schwarz, K. (2006). Microencapsulation properties of two different types of n-octenylsuccinate-derivatised starch. *European Food Research and Technology*, *222*, 155-164.

Drusch, S., Groß, N., & Schwarz, K. (2008). Efficient stabilization of bulk fish oil rich in long-chain polyunsaturated fatty acids. *European Journal of Lipid Science and Technology*, *110*, 351-359.

Drusch, S., Serfert, Y., Scampicchio, M., Schmidt-Hansberg, B., Schwarz, K. (2007). Impact of physicochemical characteristics on the oxidative stability of fish oil microencapsulated by spray-drying. *Journal of Agricultural and Food Chemistry*, *55*, 11044-51.

Dybowska, B. E. (2008). Properties of milk protein concentrate stabilized oil-in-water emulsions. *Journal of Food Engineering*, *88*, 507–512.

Dybowska, B. E. (2011). Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. *Journal of Food Engineering*, *104*, 81-88.

Ellis, R. J. (1994) Roles of molecular chaperones in protein folding. *Current Opinion in Structural Biology*, *4*, 117–122.

Elversson, J., & Millqvist-Fureby, A. (2005). Aqueous two-phase systems as a formulation concept for spray-dried protein. *International Journal of Pharmaceutics*, 294, 73−87.

Emulsions and Emulsification (2009). Retrieved November 13, 2015, from *http://www.particlesciences.com/news/technical-briefs/2009/emulsions-and-emulsification.html*

Euston, S. E., Singh, H., Munro, P. A., & Dalgleish, D. G. (1996). Oil-in-water emulsions stabilized by sodium caseinate or whey protein isolate as influenced by glycerol monostearate. *Journal of Food Science, 61,* 916-920.

Euston, S. R., & Hirst, R. L. (1999). Comparison of the concentration-dependent emulsifying properties of protein products containing aggregated and non-aggregated milk protein. *International Dairy Journal, 9,* 693−701.

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

Faldt, P., & Bergenstahl, B. (1994). The surface-composition of spray-dried protein-lactose powders. *Colloids and Surfaces A-Physicochemical and Engineering Aspects, 90,* 183−190.

Fäldt, P., & Bergenstahl, B. (1996 a). Spray-dried whey protein/lactose/soybean oil emulsions. 1. Surface composition and particle structure. *Food Hydrocolloids, 10,* 421-429.

Faldt, P., & Bergenstahl, B. (1996 b). Spray-dried whey protein/lactose/soybean oil emulsions. 2. Redispersibility, wettability and particle structure. *Food Hydrocolloids, 10,* 431−439.

Faldt, P., & Sjoholm, I. (1996). Characterization of spray-dried whole milk. *Milchwissenschaft, 51,* 88-90.

Faldt, P., Bergenstahl, B., & Carlsson, G. (1993). The surface coverage of fat on food powders analyzed by ESCA (electron spectroscopy for chemical analysis). *Food structure (USA)*.

Fang, Y., & Dalgleish, D. D. (1998). The conformation of α-lactalbumin as a function of pH, heat treatment and adsorption at hydrophobic surfaces studied by FTIR. *Food Hydrocolloids, 12*, 121–126.

Fang, Y., & Dalgleish, D. G. (1993). Dimensions of the adsorbed layers in oil-in-water emulsions stabilized by caseins. *Journal of Colloid and Interface Science, 156*, 329-334.

Faraji, H., McClements, D. J., & Decker, E. A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 52*, 4558-4564.

Farrer D, Lips A (1999) On the self assembly of sodium caseinate. *International Dairy Journal, 9*, 281-286.

Fitzpatrick, J. J., Iqbal, T., Delaney, C., Twomey, T., & Keogh, M. K. (2004). Effect of powder properties and storage conditions on the flowability of milk powders with different fat contents. *Journal of Food Engineering, 64*, 435-444.

Florence, A. T., & Whitehill, D. (1981). Some features of breakdown in water-in-oil-in-water multiple emulsions. Journal of Colloid and Interface Science, 79, 243-256.

Fox, P. F. (2001). Milk proteins as food ingredients. *International Journal of Dairy Technology, 54*, 41-55.

Fox, P. F. (2003). Milk proteins: general and historical aspects. In P.F. Fox & P. L. F. McSweeney (Eds.), *Advanced Dairy Chemistry—1 Proteins* (pp. 1-48). Verlag, US; Springer.

Freudig, B., Hogekamp, S., & Schubert, H. (1999). Dispersion of powders in liquids in a stirred vessel. *Chemical Engineering and Processing: Process Intensification, 38,* 525-532.

Friberg, S., Larsson, K., & Sjoblom, J. (Eds.). (2003). *Food emulsions*. Boca Raton, US: CRC Press.

Galazka, V. B., Dickinson, E., & Ledward, D. A. (2000). Influence of high pressure processing on protein solutions and emulsions. *Current Opinion in Colloid & Interface Science, 5,* 182-187.

Garg, M. L., Wood, L. G., Singh, H., Moughan, P. J. (2006). Means of delivering recommended levels of long chain n-3 polyunsaturated fatty acids in human diets. *Journal of Food Science, 71,* 66-71.

Gautam, A., Patrick, M., & Dagerath, M.L. (2007). WO2006058634. 15.08.2007.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40,* 1107-1121.

Golding, M., & Wooster, T. J. (2010). The influence of emulsion structure and stability on lipid digestion. *Current Opinion in Colloid & Interface Science, 15,* 90-101.

Gotoh, N., & Wada, S. (2006). The importance of peroxide value in assessing food quality and food safety. *Journal of the American Oil Chemists' Society*, 83, 473-474.

Granelli, K., Faldt, P., Appelqvist, L. A., & Bergenstahl, B. (1996). Influence of surface structure on cholesterol oxidation in model food powders. *Journal of the Science of Food and Agriculture*, *71*, 75-82.

Guyomarc'h, F., Nono, M., Nicolai, T., & Durand, D. (2009). Heat-induced aggregation of whey proteins in the presence of κ-casein or sodium caseinate. *Food Hydrocolloids*, *23*, 1103–1110.

Haque, Z., & Kinsella, J. E. (1987). Heat-induced changes in the hydrophobicity of kappa-casein and. BETA.-lactoglobulin. *Agricultural and Biological Chemistry*, *51*, 2245-2247.

Havea, P. (2006) Protein interaction in milk protein concentrate powders. *Internationa Dairy Journal, 16,* 415–422.

Havea, P., Singh, H., & Creamer, L. K. (2001). Characterization of heat-induced aggregates of β-lactoglobulin, α-lactalbumin and bovine serum albumin in a whey protein concentrate environment. *Journal of Dairy Research, 68*, 483-497.

Havea, P., Singh, H., Creamer, L. K., & Campanella, O. H. (1998). Electrophoretic characterization of the protein products formed during heat treatment of whey protein concentrate solutions. *Journal of Dairy Research*, *65*, 79–91.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001) Microencapsulating properties of whey protein concentrate 75. *Journal of Food Science*, *66*, 675–680.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal*, *11*, 137−144.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Microencapsulating properties of sodium caseinate. *Journal of Agricultural and Food Chemistry*, *49*, 1934-1938.

Hogan, S. A., McNamee, B. F., O'Riordan, E. D., & O'Sullivan, M. (2001). Microencapsulating properties of whey protein concentrate *75*. *Journal of Food Science*, *66*, 675–680.

Hogan, S. A., O'Riordan, E. D., & O'Sullivan, M. (2003). Microencapsulation and oxidative stability of spray-dried fish oil emulsions. *Journal of Microencapsulation, 20*, 675-88.

Horne, D. S. (2003). Casein micelles as hard spheres: limitations of the model in acidified gel formation. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *213*, 255-263.

Hotrum, N. E., van Vliet, T., Stuart, M. A. C., & van Aken, G. A. (2002) Monitoring entering and spreading of emulsion droplets at an expanding air/water interface: a novel technique. *Journal of Colloids and Interface Science*, *247*, 125–131.

Hunt, J. A., & Dalgleish, D. G. (1994). Adsorption behavior of whey protein isolate and caseinate in soya oil-in-water emulsions. *Food Hydrocolloids*, 8, 175–187.

Hunt, J. A., & Dalgleish, D. G. (1994). Effect of pH on the stability and surface composition of emulsions made with whey protein isolate. *Journal of Agricultural and Food Chemistry*, *42*, 2131-2135.

Hunter, R. J. (1986). *Foundations of Colloid Science*. Oxford, UK: Oxford University Press.

Jacobsen, C., Let, M. B., Nielsen, N. S., & Meyer, A. S. (2008). Antioxidant strategies for preventing oxidative flavour deterioration of foods enriched with n-3 polyunsaturated lipids: a comparative evaluation. *Trends in Food Science & Technology*, *19*, 76-93.

Jafari, S. M., Assaidpoor, E., Bhandari, B., & He, Y. H. (2008). Nano-particle encapsulation of fish oil by spray drying. *Food Research International*, *41*, 172–183.

Jafari, S. M., He, Y. H., & Bhandari, B. (2007 a). Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques. *Food Research International, 40*, 862−873.

Jafari, S. M., He, Y., & Bhandari, B. (2007 b). Production of sub-micron emulsions by ultrasound and microfluidization techniques. Journal of Food Engineering, *82*, 478-488.

Jahaniaval, F., Kakuda, Y., Abraham, V., & Marcone, M. F. (2000). Soluble protein fractions from pH and heat treated sodium caseinate: physicochemical and functional properties. *Food research international*, *33*(8), 637-647.

Jang, H. D., & Swaisgood, H. E. (1990). Disulfide bond formation between thermally denatured β-lactoglobulin and κ-casein in casein micelles. *Journal of Dairy Science, 73,* 900-904.

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

Jouppila, K., & Roos, Y. H. (1994). Glass transitions and crystallization in milk powders. *Journal of Dairy Science*, *77,* 2907-2915.

Katsuda, M. S., McClements, D. J., Miglioranza, L. H., & Decker, E. A. (2008). Physical and oxidative stability of fish oil-in-water emulsions stabilized with β-lactoglobulin and pectin. *Journal of Agricultural and Food Chemistry, 56,* 5926-5931.

Kehoe, J. J., & Foegeding, E. A. (2011). Interaction between β-casein and whey proteins as a function of pH and salt concentration. *Journal of Agricultural and Food Chemistry*, *59,* 349–355.

Kelly, P. (2011). Milk Protein Products – Milk protein concentrate. In J. W. Fuquay, P. F. Fox & P. L. H. McSweeney (Eds.), *Encyclopedia of dairy sciences (pp. 848-854).* San Diego, US: Academic Press.

Kelly, J., Kelly, P. M., & Harrington, D. (2002). Influence of processing variables on the physicochemical properties of spray dried fat-based milk powders. *Le Lait*, *82*, 401-412.

Keogh, M. K., & O'Kennedy, B. T. (1999). Milk fat microencapsulation using whey proteins. *International Dairy Journal*, *9*, 657-663.

Keogh, M. K., O'Kennedy, B. T., Kelly, J., Auty, M. A., & Kelly, P. M. (2001). Stability to oxidation of spray-dried fish oil powder microencapsulated using milk ingredients. *Journal of Food Science*, *66*, 217-24.

Keowmaneechai, E., & McClements, D. J. (2006). Influence of EDTA and citrate on thermal stability of whey protein stabilized oil-in-water emulsions containing calcium chloride. *Food Research International*, *39*, 230–239.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2002). Surface characterization of four industrial spray-dried dairy powders in relation to chemical composition, structure and wetting property. *Colloids and Surfaces B: Biointerfaces*, *26*, 197-212.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2003). On the mechanisms of surface formation and the surface compositions of industrial milk powders. *Drying Technology*, *21*, 265−278.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2005). Effect of surface composition on the flowability of industrial spray-dried dairy powders. *Colloids and Surfaces B: Biointerfaces*, *46*, 182–187.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2008). Surface composition of industrial spray-dried milk powders. 1. Development of surface composition during manufacture. *Journal of Food Engineering, 94*, 163−168.

Kim, E. H. J., Chen, X. D., & Pearce, D. (2009). Surface composition of industrial spray-dried milk powders. 2. Effects of spray drying conditions on the surface composition. *Journal of Food Engineering, 94*, 169-181.

Kiokias, S., Dimakou, C., & Oreopoulou, V. (2007). Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. *Food Chemistry, 105*, 94-100.

Kolanowski, W., Ziolkowski, M., Weißbrodt, J., Kunz, B., & Laufenberg, G. (2006). Microencapsulation of fish oil by spray drying: impact on oxidative stability. Part 1. *European Food Research and Technology, 222*, 336–342.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of head of bacteriophage-T4. *Nature, 227*, 680–685.

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

Lefèvre, T., & Subirade, M. (2003). Formation of intermolecular β-sheet structures: a phenomenon relevant to protein film structure at oil–water interfaces of emulsions. *Journal of colloid and Interface Science*, *263*(1), 59-67.

Loveday, S. M., Ye, A., Anema, S. G., & Singh, H. (2014). Tuning heat-induced colloidal aggregation of whey proteins, sodium caseinate and gum arabic:

effects of protein composition, preheating and gum arabic level. *Food Research International*, *62*, 128–136.

Lucey, J. A., Srinivasan, M., Singh, H., & Munro, P. A. (2000). Characterization of commercial and experimental sodium caseinates by multiangle laser light scattering and size-exclusion chromatography. *Journal of Agricultural and Food Chemistry*, *48*, 1610-1616.

Maher, P. G., Roos, Y. H., & Fenelon, M. A. (2014). Physicochemical properties of spray dried nanoemulsions with varying final water and sugar contents. *Journal of Food Engineering*, *126*, 113-119.

Mahmoudi, N., Mehalebi, S., Nicolai, T., Durand, D., & Riaublanc, A. (2007). Light scattering study of the structure of aggregates and gels formed by heat-denatured whey protein isolate and β-lactoglobulin at neutral pH. *Journal of Agricultural and Food Chemistry*, *55*, 3104–3111.

McClements, D. J. (2005). Theoretical analysis of factors affecting the formation and stability of multilayered colloidal dispersions. Langmuir, *21*, 9777-9785.

McClements, D. J. (2010). Emulsion design to improve the delivery of functional lipophilic components. *Annual Review of Food Science and Technology, 1*, 241−269.

McClements, D. J. (2015). Food emulsions: principles, practices, and techniques. CRC press, *8-15*.

McClements, D. J., & Weiss, J. (2005). Lipid emulsions. *Bailey's industrial oil and fat products (pp. 457-502)*, New York, US: John Wiley and Sons.

McClements, D. J., Monahan, F. J., & Kinsella, J. E. (1993). Effect of emulsion droplets on the rheology of whey protein isolate gels. *Journal of Texture Studies*, *24*, 411-422.

McKenna, A. B. (1997). Examination of whole milk powder by confocal laser scanning microscopy. *Journal of Dairy Research*, *64*, 423−432.

McKenna, A. B., Lloyd, R. J., Munro, P. A., & Singh, H. (1999). Microstructure of whole milk powder and of insolubles detected by powder functional testing. *Scanning Volume*, *21*, 305–315.

McKenzie, H. A., & Sawyer, W. H. (1967). Effect of pH on beta-lactoglobulins. *Nature*, *214*, 1101-1104.

McKinnon, I. R., Yap, S. E., Augustin, M. A., & Hemar, Y. (2009). Diffusing-wave spectroscopy investigation of heated reconstituted skim milks containing calcium chloride. *Food Hydrocolloids*, *23*(4), 1127-1133.

McManus, A., Merga, M., & Newton, W. (2011). Omega-3 fatty acids. What consumers need to know. *Appetite*, *57*, 80-83.

McNamee, B. F., O'Riorda, E. D., & O'Sullivan, M. (1998). Emulsification and microencapsulation properties of gum arabic. *Journal of Agricultural and Food Chemistry*, *46*, 4551-4555.

McSweeney, S. L., Mulvihill, D. M., & O'Callaghan, D. M. (2004). The influence of pH on the heat-induced aggregation of model milk protein ingredient systems and model infant formula emulsions stabilized by milk protein ingredients. *Food Hydrocolloids*, *18*, 109-125.

Mehalebi, S., Nicolai, T., & Durand, D. (2008). Light scattering study of heat-denatured globular protein aggregates. *International Journal of Biological Macromolecules*, 43, 129–135.

Miao, S., & Roos, Y. H. (2004). Nonenzymatic browning kinetics of a carbohydrate-based low-moisture food system at temperatures applicable to spray drying. *Journal of Agricultural and Food Chemistry, 52*, 5250-5257.

Millqvist-Fureby, A., Elofsson, U., & Bergenstahl, 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*, 47−58.

Monahan, F. J., McClements, D. J., & German, J. B. (1996). Disulfide-mediated Polymerization Reactions and Physical Properties of Heated WPI-stabilized Emulsions. *Journal of Food Science, 61*, 504-509.

Moreau, D. L., & Rosenberg, M. (1996). Oxidative stability of anhydrous milkfat microencapsulated in whey proteins. *Journal of Food Science, 61*, 39-43.

Morr, C. V. (1982). Functional properties of milk proteins and their use as food ingredients. *Developments in Dairy Chemistry, 1*, 375-399.

Mounsey, J. S., & O'Kennedy, B. T. (2007). Conditions limiting the influence of thiol-disulphide interchange reactions on the heat-induced aggregation kinetics of β-lactoglobulin. *International Dairy Journal, 17,* 1034–1042.

Mulvihill, D. M., & Ennis, M. P. (2003). Functional milk proteins: Production and utilization. In P. F. Fox & P. L. H. McSweeney (Eds.), *Advanced Dairy Chemistry—1 Proteins (pp. 1175-1228)*. New York, US: Springer.

Mulvihill, D. M., & Fox, P. F. (1989). Physico-chemical and functional properties of milk proteins. In P. F. Fox (Ed.), *Developments in dairy chemistry - Volume 4. Functional milk proteins (pp.* 131-172). Philadelphia: US*:* Elsevier Inc.

Mulvihill, D. M., & Murphy, P. C. (1991). Surface active and emulsifying properties of caseins/caseinates as influenced by state of aggregation. *International Dairy Journal*, *1*, 13-37.

Murphy, J. M., & Fox, P. F. (1991). Functional properties of αs-κ-or β-rich casein fractions. *Food chemistry*, *39*(2), 211-228.

Murrieta-Pazos, I., Gaiani, C., Galet, L., Calvet, R., Cuq, B., & Scher, J. (2012) Food powders: surface and form characterization revisited. *Journal of Food Engineering, 112*, 1–21

Newton, I.S. (2001). Long-chain fatty acids in health and nutrition. In F. Shahidi & J. S. Finley (Eds.), *Omega-3 Fatty Acids: Chemistry, Nutrition, and Health Effects (pp 14-27)*. Oxford, UK; Oxford University Press.

Nicolai, T., Britten, M., & Schmitt, C. (2011). beta-Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids,* 25, 1945–1962.

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.

O'Kennedy, B. T., & Mounsey, J. S. (2006). Control of heat-induced aggregation of whey proteins using casein. *Journal of Agricultural and Food Chemistry, 54,* 889–893.

Oldfield, D. J., Singh, H., Taylor, M. W., & Pearce, K. N. (2000). Heat-induced interactions of β-lactoglobulin and α-lactalbumin with the casein micelle in pH-adjusted skim milk. *International Dairy Journal*, *10*, 509-518.

Phillips, M. C. (1981). Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. *Food Technology*, *35*, 50.

Rennie, P. R., Chen, X. D., Hargreaves, C., & Mackereth, A. R. (1999). A study of the cohesion of dairy powders. *Journal of Food Engineering*, *39*, 277-284.

Ries, D. (2009). Studies on the antioxidant activity of milk proteins in model oil-in-water emulsions: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology, Massey University, Palmerston North, New Zealand.

Risch, S. J., & Reineccius, G. (1988). Flavor encapsulation. In S. J. Risch & G. Reineccius (Eds.). *ACS symposium series (pp. 1-37)* Washington, US: American Chemical Society.

Robins, M. M., Watson, A. D., & Wilde, P. J. (2002). Emulsions - creaming and rheology. *Current Opinion in Colloid and Interface Science*, *7*, 419-425.

Ross, Y., & Karel, M. (1991). Plasticizing effect of water on thermal behavior and crystallization of amorphous food models. *Journal of Food Science*, *56*, 38-43.

Rosenberg, M., & Young, S. L. (1993). Whey proteins as microencapsulating agents. Microencapsulation of anhydrous milkfat. Structure evaluation. *Food Structure*, *12*, 31-41.

Sanchez, C. C., & Patino, J. M. R. (2005). Interfacial, foaming and emulsifying characteristics of sodium caseinate as influenced by protein concentration in solution. *Food Hydrocolloids, 19*, 407–416.

Sarkar, A. (2010). Behaviour of milk protein-stabilized oil-in-water emulsions in simulated physiological fluids: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Technology at Massey University, Palmerston North, New Zealand.

Schubert, H. (1993). Instantization of powdered food products. *International Chemical Engineering*, *33*, 28-45.

Schuck, P. (2002). Spray drying of dairy products: state of the art. *Le Lait*, *82*, 375-382.

Schuck, P., & Dolivet, A. (2002). Lactose crystallization: determination of α-lactose monohydrate in spray-dried dairy products. *Le Lait*, *82*, 413-421.

Schuck, P., Mejean, S., Dolivet, A., & Jeantet, R. (2005). Thermohygrometric sensor: a tool for optimizing the spray drying process. *Innovative Food Science & Emerging Technologies*, *6*, 45-50.

Serfert, Y., Drusch, S., & Schwarz, K. (2009). Chemical stabilization of oils rich in long-chain polyunsaturated fatty acids during homogenization, microencapsulation and storage, *Food Chemistry*, *113,* 1106-1112.

Serfert, Y., Drusch, S., & Schwarz, K. (2010). Sensory odour profiling and lipid oxidation status of fish oil and microencapsulated fish oil. *Food Chemistry, 123,* 968-975.

Shantha, N. C., & Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *Journal of AOAC International, 77,* 421-4.

Shen, Z., Rusli, J. K., Sanguansri, L., & Augustin, M. A. (2007). Retention of propanal in protein-stabilised tuna oil-in-water emulsions. *Food Chemistry, 1012,* 746-752.

Sheu, T. Y., & Rosenberg, M. (1995). Microencapsulation by spray drying ethyl caprylate in whey protein and carbohydrate wall systems. *Journal of Food Science, 60,* 98-103.

Singh, H. (2005). Milk protein functionality in food colloids. In: E. Dickinson (Ed.), *Food colloids: interactions, microstructure and processing (pp. 179-193)*. Cambridge, UK: Royal Society of Chemistry.

Singh, A. M., & Dalgleish, D. G. (1998). The emulsifying properties of hydrolyzates of whey proteins. *Journal of Dairy Science, 81,* 918-924.

Singh, H. (2005). Milk protein functionality in food colloids. S*pecial Publication-Royal Society of Chemistry, 298,* 179.

Singh, H. (2011). Aspects of milk-protein-stabilised emulsions. *Food Hydrocolloids,* 25, 1938-1944.

Singh, H., & Fox, P. F. (1995). Heat-induced changes in casein, including interactions with whey proteins. Heat-induced changes in milk., (Ed. 2), 86-104.

Singh, H., Zhu, X.Q., & Ye, A. (2006). WO2006/115420A1. 02.11.2006.

Singh, H., & Ye, A. (2009). Interactions and functionality of milk proteins in food emulsions. In A. Thompson, M. Boland, H. Singh (Eds.), *Milk proteins: from expression to foods (pp. 321−345*). London, UK : Elsevier Inc.

Singh, H., Newstead, D. F., & Fox, P. F. (1992). Aspects of proteins in milk powder manufacture. *Advanced dairy chemistry-1: Proteins.*, (Ed. 2), 735-765.

Singh, H., Tamehana, M., Hemar, Y., & Munro, P. A. (2003). Interfacial compositions, microstructure and stability of oil-in-water emulsions formed with mixtures of milk proteins and κ-carrageenan: 2. Whey protein isolate (WPI). *Food Hydrocolloids*, *17*, 549-561.

Skelbaek, T., & Andersen, S. (1994). WO94/01001. 20.01.1994.

Sliwinski, E. L., Lavrijsen, B. W. M., Vollenbroek, H. J., van der Stege, H. J., van Boekel, M. A. J. S., & Wouters, J. T. M. (2003a). Effects of spray drying on the physicochemical properties of milk protein-stabilized emulsions. *Colloids and Surfaces B: Biointerfaces*, *31*, 219–229.

Sliwinski, E. L., Roubos, P. J., Zoet, F. D., Van Boekel, M. A. J. S., & Wouters, J. T. M. (2003). Effects of heat on physicochemical properties of whey protein-stabilised emulsions. *Colloids and Surfaces B: Biointerfaces*, *31*, 231-242.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohkawara, M., & Linko, P. (2003). Microencapsulation by spray drying: influence of emulsion size on the retention of volatile compounds. *Journal of Food Science*, *687*, 2256-2262.

Spray Dryer MSD™ Spray Dryer. (2015). Retrieved November 14, 2015, from *http://www.gea.com/global/en/products/gea-msd.jsp*.

Srinivasan, M., Singh, H., & Munro, P. A. (2003) Influence of retorting (121 °C for 15 min), before or after emulsification, on the properties of calcium caseinate oil-in-water emulsions. *Food Chemistry, 80*, 61–69.

Srinivasan, M., Singh, H., & Munro, P. A. (1996). Sodium caseinate-stabilized emulsions: factors affecting coverage and composition of surface proteins. *Journal of Agricultural and Food Chemistry*, *44*, 3807-3811.

Srinivasan, M., Singh, H., & Munro, P. A. (1999). Adsorption behaviour of sodium and calcium caseinates in oil-in-water emulsions. *International Dairy Journal*, *9*, 337-341.

Srinivasan, M., Singh, H., & Munro, P. A. (2001). Creaming stability of oil-in-water emulsions formed with sodium and calcium caseinates. *Journal of Food Science*, *66*, 441–446.

Surh, J., Ward, L. S., & McClements, D. J. (2006). Ability of conventional and nutritionally-modified whey protein concentrates to stabilize oil-in-water emulsions. *Food Research International*, *39*, 761–771.

Swaisgood, H. E. (1982). Chemistry of milk protein. *Developments in Dairy Chemistry*, *1*, 1-59.

Taneja, A., & Singh, H. (2012). Challenges for the delivery of long-chain n-3 fatty acids in functional foods. *Annual Review of Food Science and Technology*, *3*, 105–123.

Taneja, A., & Singh, H. (2012). Challenges for the delivery of long-chain n-3 fatty acids in functional foods. *Annual review of food science and technology*, *3*, 105-123.

Taneja, A., Ye, A., & Singh, H. (2015). Influence of protein concentration on the stability of oil-in-water emulsions formed with aggregated milk proteins during spray drying. *Dairy Science & Technology*, *95*, 279–293.

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.

Taylor, P. (1998). Ostwald ripening in emulsions. *Advances in colloid and interface science*, *75*, 107-163.

Teonou, E., & Fitzpatrick, J. J. (1999). Effect of relative humidity and temperature on food powder flowability. *Journal of Food Engineering*, *42*, 109-116.

Tesch, S., & Schubert, H. (2002). Influence of increasing viscosity of the aqueous phase on the short-term stability of protein stabilized emulsions. *Journal of Food Engineering*, *52*, 305−312.

Tolkach, A., & Kulozik, U. (2005). Effect of pH and temperature on the reaction kinetic parameters of the thermal denaturation of b-lactoglobulin. *Milchwissenschaft*, *60*, 249–252.

Tong, L. M., Sasaki, S., McClements, D. J., & Decker, E. A. (2000). Mechanisms of the antioxidant activity of a high molecular weight fraction of whey. *Journal of Agricultural and Food Chemistry*, *48*, 1473-1478.

Tornberg, E., & Hermansson, A. M. (1977). Functional characterization of protein stabilized emulsions: effect of processing. *Journal of Food Science*, *42*, 468-472.

Tuinier, R., & De Kruif, C. G. (1999). Phase separation, creaming, and network formation of oil-in-water emulsions induced by an exocellular polysaccharide. *Journal of Colloid and Interface Science*, *218*, 201-210.

Tuinier, R., Dhont, J. K. G., & De Kruif, C. G. (2000). Depletion-induced phase separation of aggregated whey protein colloids by an exocellular polysaccharide. Langmuir, *16*, 1497-1507.

Tuinier, R., Ten Grotenhuis, E., Holt, C., Timmins, P. A., & De Kruif, C. G. (1999). Depletion interaction of casein micelles and an exocellular polysaccharide. *Physical Review E*, *60*, 848.

Twomey, M., Keogh, M. K., O'Kennedy, B. T., Auty, M., & Mulvihill, D. M. (2000). Effect of milk composition on selected properties of spray-dried high-fat and skim-milk powders. *Irish Journal of Agricultural And Food Research*, 79-94.

van Aken, G. A. (2004). Coalescence mechanisms in protein-stabilized emulsions. In S.E. Friberg, K. Larsson, & J. Sjoblom (Eds.), *Food Emulsions (pp. 299-329)*. New York, US: Marcel Dekker.

van Aken, G. A., Blijdenstein, T. B., & Hotrum, N. E. (2003). Colloidal destabilisation mechanisms in protein-stabilised emulsions. *Current Opinion in Colloid and Interface science*, *8*, 371-379.

Vasbinder, A. J., & de Kruif, C. G. (2003). Casein–whey protein interactions in heated milk: the influence of pH. *International Dairy Journal*, *13*, 669-677.

Vega, C., Goff, D. H., & Roos, Y. H. (2007) Casein molecular assembly affects the properties of milk fat emulsions encapsulated in lactose or trehalose matrices. *International Dairy Journal*, *17*, 683–695.

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

Vega, C., Kim, E. H. J., Chen, X. D., & Roos, Y. H. (2005). Solid-state characterization of spray-dried ice cream mixes. *Colloids and Surfaces B: Biointerfaces*, *45*, 66-75.

Verdurmen, R. E. M., Jong, P. D., & Smit, G. (2003). Optimising product quality and process control for powdered dairy products. *Dairy Processing: Improving Quality*, 333-365.

Lara-Villoslada, F., Olivares, M., & Xaus, J. (2005). The balance between caseins and whey proteins in cow's milk determines its allergenicity. *Journal of dairy science*, *88*, 1654-1660.

Walstra, P. (1987). Overview of emulsion and foam stability. *Food Emulsions and Foams*, 242-257.

Walstra, P. (1993). Principles of emulsion formation. In P. Walstra (Ed.), *Chemical Engineering Science (pp. 333-349)*. Basel, CH: Marcel Dekker.

Walstra, P. (2003). Changes in dispersity. In P. Walstra (Ed.), *Physical chemistry of foods* (pp. 476−547). Basel, CH: Marcel Dekker.

Wang, R., Tian, Z., & Chen, L. (2011). A novel process for microencapsulation of fish oil with barley protein. *Food Research International*, *44*, 2735-2741.

Ye, A. Q., Srinivasan, M., & Singh, H. (2000) Influence of NaCl addition on the properties of emulsions formed with commercial calcium caseinate. *Food Chemistry*, *69*, 237–244.

Ye, A. (1999). Influence of calcium chloride addition on the properties of emulsions formed with milk protein products: a thesis presented in partial fulfilment of the requirements for the degree of master of technology, Massey University, Palmerston North, New Zealand.

Ye, A. Q. (2008). Interfacial composition and stability of emulsions made with mixtures of commercial sodium caseinate and whey protein concentrate. *Food Chemistry*, *110*, 946–952.

Ye, A. Q. (2010). Surface protein composition and concentration of whey protein isolate-stabilized oil-in-water emulsions: effect of heat treatment. *Colloids and Surfaces B: Biointerfaces*, *78*, 24–29.

Ye, A. Q. (2011). Functional properties of milk protein concentrates: emulsifying properties, adsorption and stability of emulsions. *International Dairy Journal*, *21*, 14−20.

Ye, A., & Singh, H. (2000). Influence of calcium chloride addition on the properties of emulsions stabilized by whey protein concentrate. *Food Hydrocolloids, 14*, 337–346.

Ye, A., Anema, S. G., & Singh, H. (2007). Behaviour of homogenized fat globules during the spray drying of whole milk. *International Dairy Journal, 17*, 374−382.

Young, S. L., Sarda, X., & Rosenberg, M. (1993). Microencapsulating properties of whey proteins. 1. Microencapsulation of anhydrous milk fat. *Journal of Dairy Science*, *76*(10), 2868-2877.

Ziegler, G. R., & Foegeding, E. A. (1990). The gelation of proteins. *Advances in food and nutrition research*, *34*, 203-298.

Permissions

RightsLink

Copyright Clearance Center

Thank You For Your Order!

Dear Mr. Amit Taneja,

Thank you for placing your order through Copyright Clearance Center's RightsLink service. Elsevier has partnered with RightsLink to license its content. This notice is a confirmation that your order was successful.

Your order details and publisher terms and conditions are available by clicking the link below:
http://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=9fa42a84-32e0-45c2-8c89-b4733116984b

Order Details
Licensee: Amit Taneja
License Date: Feb 13, 2016
License Number: 3807290192026
Publication: International Dairy Journal
Title: Behaviour of oil droplets during spray drying of milk-protein-stabilised oil-in-water emulsions
Type Of Use: reuse in a thesis/dissertation
Total: 0.00 USD

To access your account, please visit https://myaccount.copyright.com.

Please note: Online payments are charged immediately after order confirmation; invoices are issued daily and are payable immediately upon receipt.

To ensure that we are continuously improving our services, please take a moment to complete our customer satisfaction survey.

B.1:v4.2

+1-855-239-3415 / Tel: +1-978-646-2777
customercare@copyright.com
http://www.copyright.com

RightsLink

Copyright Clearance Center

Thank You For Your Order!

Dear Mr. Amit Taneja,

Thank you for placing your order through Copyright Clearance Center's RightsLink service. Springer has partnered with RightsLink to license its content. This notice is a confirmation that your order was successful.

Your order details and publisher terms and conditions are available by clicking the link below:
http://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=b0fd63fb-b5e1-4633-b052-5585dd1abb6b

Order Details
Licensee: Amit Taneja
License Date: Feb 13, 2016
License Number: 3807290556814
Publication: Dairy Science & Technology
Title: Influence of protein concentration on the stability of oil-in-water emulsions formed with aggregated milk proteins during spray drying
Type Of Use: Thesis/Dissertation
Total: 0.00 USD

To access your account, please visit https://myaccount.copyright.com.

Please note: Online payments are charged immediately after order confirmation; invoices are issued daily and are payable immediately upon receipt.

To ensure that we are continuously improving our services, please take a moment to complete our customer satisfaction survey.

B.1:v4.2

+1-855-239-3415 / Tel: +1-978-646-2777
customercare@copyright.com
http://www.copyright.com