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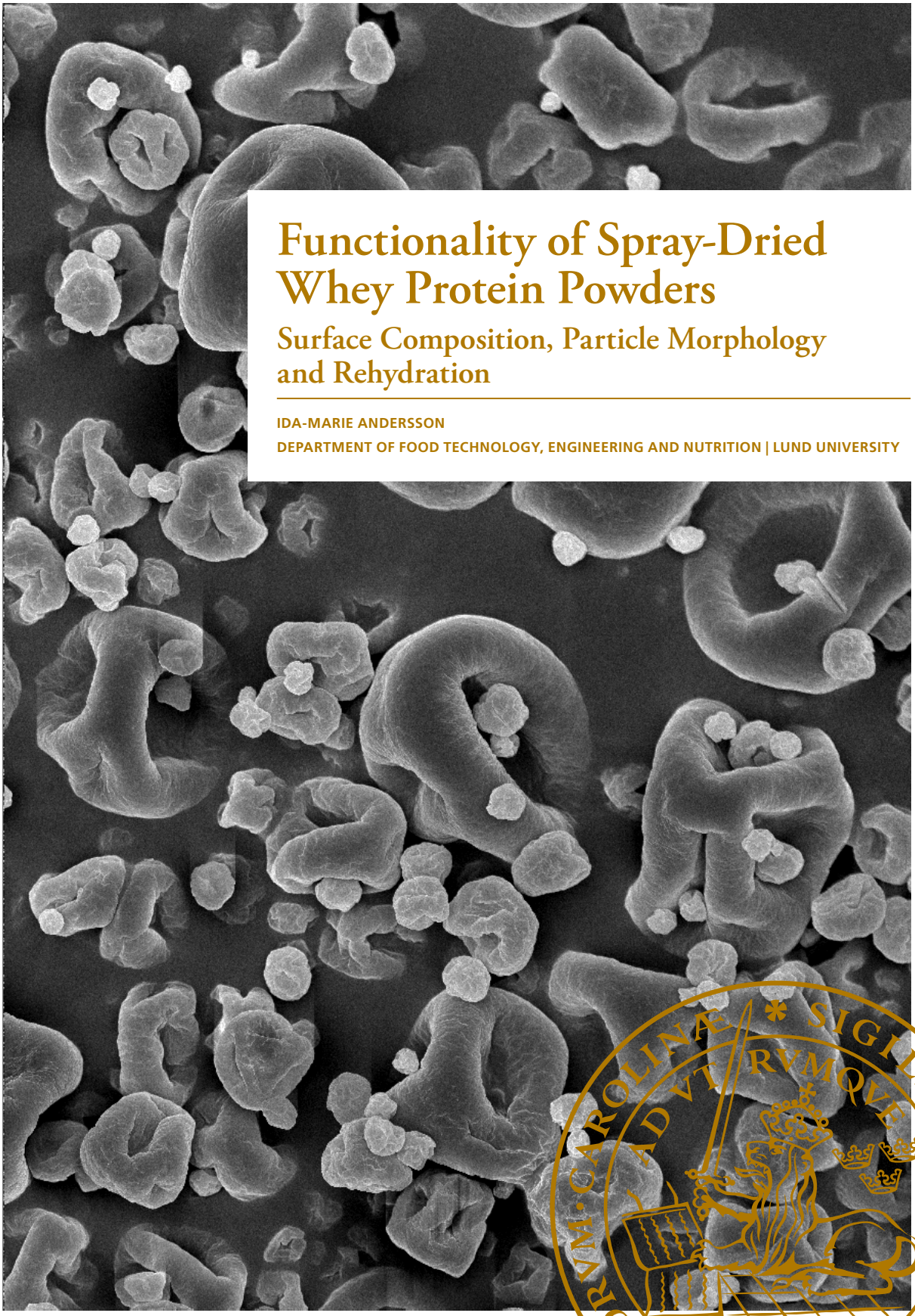
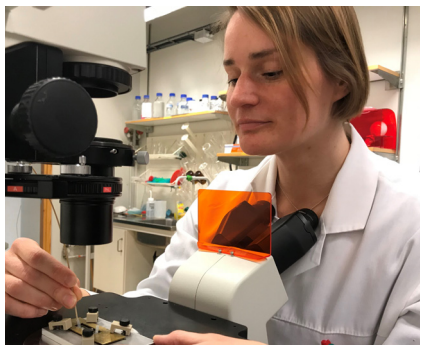


Functionality of Spray-Dried Whey Protein Powders

Surface Composition, Particle Morphology and Rehydration

IDA-MARIE ANDERSSON
DEPARTMENT OF FOOD TECHNOLOGY, ENGINEERING AND NUTRITION | LUND UNIVERSITY



Ida-Marie Andersson has a MSc in Engineering, Biotechnology with specialization in food technology from Lund University Lund, Sweden. Her doctoral studies have been in collaboration between the Department of Food Technology, Engineering and Nutrition at Lund University and Arla Foods Ingredients, Nr Vium, Denmark.

The aim of her doctoral thesis was to evaluate the effect of the feed properties and how that affects surface composition, particle morphology and rehydration properties of spray-dried whey protein powders with varying ratios of lactose. For powders, it is essential that they are easily dispersed and dissolved in order to fulfil the specified nutrient content and the functionality in the final product. Poor rehydration can cause challenges on an industrial level as well as for the consumers. This thesis has shown that changes in feed composition as well as the physical state of milk serum proteins (native vs. aggregated) did not have a major impact on the rehydration properties and particle morphology, even when a large fraction of the proteins were aggregated. Based on these observations, it is suggested that the particle surface is dominated by native proteins and that protein aggregates are mainly found in the interior of the powder particle. Thus, this thesis has shown that native whey proteins and lactose have the potential to protect less surface-active components, such as protein aggregates, in the powder particle. This knowledge can be used to formulate powders with improved rehydration characteristics.

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LUND UNIVERSITY

DOCTORAL DISSERTATION

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Functionality of Spray-Dried Whey Protein Powders- Surface Composition, Particle Morphology and Rehydration

Abstract

Whey protein powder functionality is expected to be closely linked to both structure and properties of the proteins. It is essential that whey protein powders are easily dispersed and dissolved in order to fulfil the specified nutrient content and the functionality in the final product. Poor rehydration can cause challenges on an industrial level as well as for the consumers. In this thesis, several studies were carried out, examining the effects of varying the composition in the feed on surface properties, particle morphology and functional properties of spray-dried powders. A membrane filtered product, serum protein concentrate (SPC), with a high fraction of native proteins, was studied with varying lactose content using different techniques.

For SPC/lactose (% w/w) systems, the stiffness of the interface of the feed droplet had an impact on the particle morphology. Feed droplets with a high modulus of elasticity and, thus, a stiff interface resulted in particles with thick ridges and deep dents. Systems with a low modulus of elasticity resulted in particles that were either smooth or covered with a high frequency of dents and thin ridges. The time needed to obtain wettability of these powders showed a positive relationship with the protein surface coverage, which was estimated by X-ray photoelectron spectroscopy (XPS). Microstructural investigations of the internal structures of the particles with confocal Raman microscopy revealed that the protein-rich domain in the vicinity of the powder particle tended to become thinner as the bulk protein concentration increased in the powders. This suggests that the protein surface coverage has a more important role for the wettability than the thickness of the protein layer.

Scanned electron microscopy images revealed a similar particle morphology as the fraction of native proteins decreased from 100 to 45% as a result of heat treatment of the feed. However, the interior of the particle showed large differences where protein aggregates could be distinguished. The results imply that the surface composition was rather similar. In addition, the rehydration properties of these powders were not affected to a large extent by the protein denaturation. However, in serum protein/lactose 40/60 (% w/w) powders with a large fraction of aggregated proteins (95%), it was observed that an addition of native proteins improved the wettability. Further, the results from the XPS indicated that the powders with <15% native protein had approximately 10-15% of denatured/aggregated proteins at the particle surface which could explain the poorer wettability of these powders.

Lactosylation of the native protein fraction had no effect on the rehydration properties of serum protein/lactose (% w/w) 1/99 and 60/40 powders even though the degree of lactosylation increased from 10 to 35% in some of the powders after storage (30°C, aw 0.23 for 25 days). On the other hand, it was observed that lactosylation was more pronounced in powders with a high fraction of proteins and not in the powders with a high fraction of lactose. It is suggested that the rate of lactosylation is higher in protein-rich domains with dissolved lactose than in lactose-rich domains with dissolved proteins.

Even though the feed was subjected to severe heat treatment and a large fraction of the protein aggregated, the rehydration properties and the particle morphology of the spray-dried powder was not affected to a large extent. Thus, as long as there are native proteins present in the system, they tend to dominate the particle surface and thereby protect the denatured and aggregated proteins, which are mainly found in the interior of the powder particles. This insight has relevance for formulation of whey powders with improved rehydration properties.

Key words

Milk serum protein, lactose, whey protein powders, spray drying, particle morphology, surface composition, rehydration and functionality

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MADE IN SWEDEN

At least I made it my whey

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List of publications

This thesis is based on the following publications, which will be referred to in the text by their Roman numerals. The publications are appended in the end of the thesis.

I. Andersson I.M., Glantz M., Alexander M., Millqvist-Fureby A., Paulsson M., & Bergenståhl B. (2018), **Impact of surface properties on morphology of spray-dried milk serum protein/lactose systems**, *International Dairy Journal*, 85; 86-95

II. Andersson I.M., Millqvist-Fureby A., Sommertune J., Alexander M., Hellström N., Glantz M., Paulsson M. & Bergenståhl B. (2019), **Impact of protein surface coverage and layer thickness on rehydration characteristics of milk serum protein/lactose powder particles**, *Colloids and Surfaces A*, 561; 395-404

III. Andersson I.M, Alexander M., Paulsson M., Glantz M. & Bergenståhl B., **Effect of feed composition, protein denaturation and storage of milk serum protein/lactose powders on lactosylation**, *Under review*

IV. Andersson I.M., Alexander M., Paulsson M., Glantz M. & Bergenståhl B., **Effect of feed composition, protein denaturation and storage of milk serum protein/lactose powders on rehydration characteristics**, *Under review*

V. Andersson I.M., Millqvist-Fureby A., Alexander M., Paulsson M., Glantz M & Bergenståhl B., **Particle morphology and rehydration properties of spray-dried microgels and fractal aggregates with varying fraction of native milk serum proteins**, *Manuscript*

The author's contribution to the papers

I. The author designed the study with the co-authors, performed the major part of the experimental work. X-ray photoelectron spectroscopy (XPS) was performed as an analytical service at RISE, Research Institute of Sweden, Stockholm. Scanned electron microscopy was performed as an analytical service at Centre for Analysis and Synthesis (CAS), Lund University, Sweden. The results were evaluated together with the co-authors. The author wrote the first draft of the paper, which was revised by the co-authors.

II. The author designed the study together with the co-authors and performed the major part of the experimental work. Jens Sommertune at RISE Research Institute of Sweden performed the confocal Raman microscopy. Scanned electron microscopy was performed as an analytical service at CAS (Lund University, Sweden). The results were evaluated together with the co-authors. The author wrote the first draft of the paper, which was revised by the co-authors.

III. The author designed the study together with the co-authors, performed the major part of the experimental work. As an analytical service, ultra-high performance liquid chromatography (U-HPLC) and liquid chromatography mass-spectrometry were performed at Arla Foods Ingredients, Nr. Vium, Denmark. The author analysed the results together with the co-authors and wrote the first draft of the paper, which was revised by the co-authors.

IV. The author designed the study together with the co-author, performed the experimental work, analysed the results together with the co-author and wrote the first draft of the paper, which was revised by the co-authors.

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Contribution to conferences and workshops

Andersson I.M., Alexander M., Millqvist-Fureby A., Paulsson M., Glantz M. & Bergenståhl B. (Oral presentation), How does heat exposure of the feed affect the dissolution rate of spray-dried milk serum protein/lactose powders?, 11th NIZO Dairy Conference, October 8-11, 2019, Papendal, The Netherlands

Andersson I.M., Millqvist-Fureby A., Sommertune J., Alexander M., Hellström N., Glantz M., Paulsson M. & Bergenståhl B. (Oral presentation), Surface and internal chemical characterization of dairy powder particles, Confocal Correlative Raman Imaging Workshop at RISE, November 5-6, 2018, Stockholm, Sweden

Andersson I.M, Hellström N., Sommertune J., Glantz M., Alexander M., Holm Nielsen J., Millqvist-Fureby A., Paulsson M. & Bergenståhl B. (Poster presentation), Impact of surface properties and internal distribution of powder constituents on rehydration characteristics of spray-dried milk serum protein powders, 17th Food Colloids Conference, April 8-11, 2018, Leeds, United Kingdom

Andersson I.M., Glantz M., Alexander M., Millqvist-Fureby A., Paulsson M. & Bergenståhl B. (Oral presentation), Rehydration and functionality of dairy powders – Impact of Surface Properties on Morphology of Spray-Dried Milk Serum Protein-lactose systems, Workshop Nordic-Baltic Dairy Network – Milk composition – Functional Genomics and Health Aspects, November 20-22, 2017, Larkollen, Norway

Andersson I.M., Hellström N., Glantz M., Alexander M., Holm Nielsen J., Paulsson M. & Bergenståhl B. (Oral presentation), Rehydration and functionality of dairy powders, Arla Foods Ingredients, August 24, 2017, Nr Vium, Denmark

Andersson I.M., Hellström N., Glantz M., Alexander M., Holm Nielsen J., Paulsson M. & Bergenståhl B. (Oral presentation), Impact of protein/lactose ratio on the dissolution rate of spray-dried powders, Nordic Dairy Congress, June 7-9, 2017, Copenhagen, Denmark

Andersson I.M., Hellström N., Glantz M., Alexander M., Holm Nielsen J., Paulsson M. & Bergenståhl B. (Poster presentation), Impact of protein/lactose ratio on the dissolution rate of spray-dried powders, IDF World Dairy Summit, October 16-21, 2016, Rotterdam, The Netherlands

Abstract

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Populärvetenskaplig sammanfattning

Vasslepulver – torrt pulver med spännande upplösning

Vasslepulver är en näringsrik produkt med högt proteininnehåll. Det används flitigt som tillskott vid träning och modersmjölksersättning, men även som tillsats i en mängd livsmedel för att bidra till bättre textur och konsistens. Det är inte helt oproblematiskt att lösa upp pulvret. Som konsument har man säkerligen upplevt mer än en gång att det bildas klumpar när man försöker lösa upp det, vilket kan försvåra näringsupptaget samt att munkänslan blir mindre trevlig. Dessutom skapar det utmaningar för industrin. För att pulvret ska lösas upp måste det först vätas av vätskan som man blandar ner pulvret i. För att ta ett exempel; när man blandar en proteinshake används ca 25 gram pulver, eller med andra ord 20 miljoner pulverpartiklar. Den totala ytan av dessa partiklar är närmare 40 kvadratmeter, samma yta som en större enrumslägenhet! Och det är just vid vätningen som problem kan uppstå då ytan på partiklarna väts av vätskan som då blir klibbig, vilket kan göra att flera partiklar klibbar samman och på så vis bildar klumpar. Men vad är det då som påverkar vätningen och upplösningen av pulvret? Men vi börjar från början, vad är egentligen vassle?

Förr ansågs vassle från osttillverkning vara en oanvändbar produkt och mejerierna gav mer eller mindre bort vasslen till bönder för att användas som foder till kreaturen. Tack vare utvecklingen av industriella tekniker, såsom filtrering och spraytorkning, gjordes det möjligt att ta tillvara på vasslen. Idag är produkten vassle lika högt värderad som själva osten. Vassle består mestadels av vatten men innehåller cirka 1 % protein, 4 % laktos och lite salter. För att skapa mervärde till produkten filtreras laktosen bort och sedan spraytorkas vasslen till ett fint pulver. Vid spraytorkning finfördelas vasslen i små fina droppar som torkas med hetluft. Från droppe till pulverpartikel tar det bara några sekunder. Att torka vasslen har en mängd fördelar såsom att hållbarheten förlängs eftersom bakterier inte trivs i torra miljöer, produkten kan förvaras i rumstemperatur, mängden protein per kilo produkt är hög och transportkostnaderna reduceras kraftigt vilket är positivt för miljön. Vad som gör vasslepulver så intressant är just dess proteiner då de innehåller en mängd livsnödvändiga aminosyror (proteiners byggstenar) för kroppen och att proteinerna snabbt tas upp av kroppen.

Idag produceras det, genom spraytorkning, över 2 miljoner ton vasslepulver bara i EU och siffran väntas stiga med åren. En intressant fråga som uppstår är hur vassleproteinerna påverkas av spraytorkningen och hur det i sin tur påverkar upplösningen av pulvret. Det är här som den här studien kommer in i bilden. I den här studien har spraytorkade vassleproteinpulver med varierande laktoshalt undersökts. Proteiner är ytaktiva, vilket innebär att de söker sig till ytan av en

vattendroppe. Under spraytorkningens första del, när vasslen finfördelas i små droppar, söker sig alltså proteinerna till ytan innan själva pulverpartikeln bildas.

Resultaten visade att ytan på pulverpartiklarna spelar stor roll för vätningen. Ju mer protein vid ytan, desto längre tid tar det för vattnet att väta partikeln. Dessutom visade resultaten att proteinlagret vid ytan inte nödvändigtvis blev tjockare ju mer protein som fanns i vasslen som spraytorkades. Förutom skillnader i vätningen visade denna avhandling att utseendet på pulverpartiklarna ändras beroende på förhållandet mellan protein och laktos. Med andra ord kan utseendet av pulverpartikeln säga något om sammansättningen av ytan. En annan intressant upptäckt som gjorts är att värmebehandling av vasslen innan torkning påverkar utseendet av pulverpartiklarna, men också vätningen som tar något längre tid. Det indikerar på att sammansättningen av pulverytan har ändrats. Värmning är ett nödvändigt steg innan torkning för att avdöda värmekänsliga mikroorganismer som förekommer i vasslen. Proteinerna i vasslen är också värmekänsliga och kan ändra sin struktur vilket kan göra att de blir mindre benägna att komma i kontakt med vatten. Ett nativt protein, alltså ett protein som har sin naturliga struktur, väts oftast lättare jämfört med ett protein som påverkats av värmebehandling. Vad händer om man tillsätter nativt protein till vassle som värmebehandlats kraftigt? Jo, vätningen av pulverpartiklarna går snabbare eftersom det nativa proteinet snabbare tar sig till pulverpartikelytan än proteinerna som påverkats av uppvärmningen.

Denna avhandling har bidragit med ytterligare kunskap om vad som påverkar vätningen och upplösningen av vasslepulver. Tack vare den här forskningen är vi ett steg närmare en snabb upplösning på ett svårlösligt problem.

Abbreviations

a_w	Water activity
α-la	α-lactalbumin
β-lg	β-lactoglobulin
BSA	Bovine serum albumin
CRM	Confocal Raman microscopy
Cryo-TEM	Cryo-transmission electron microscopy
MPC	Milk protein concentrate
SEM	Scanned electron microscopy
SMP	Skim milk powder
SPC	Serum protein concentrate
WP	Whey proteins
WPC	Whey protein concentrate
WPH	Whey protein hydrolysate
WPI	Whey protein isolate
XPS	X-ray photoelectron spectroscopy

Introduction

In 2018, almost 13 million tonnes of dairy powders were produced worldwide (IDF, 2019). Of these, whey powders accounted for more than three million tonnes where nearly 70% is produced in the European Union (IDF, 2019). During the last five years, the whey powder production has increased with 11% and is expected to continue to grow worldwide thanks to opportunities related to nutritious foods, infant formulas and medical use (IDF, 2019). Around 80% of the whey powder is produced from liquid whey which is a product from cheese manufacturing, and the rest is derived from casein production.

Rehydration and functionality of spray-dried whey powders are important quality parameters in industrial applications, either as ingredient in various types of food or in dry blend products such as performance products and infant formulas. It is crucial that the powder is easily dispersed and dissolved in order to fulfil the specified nutrient content and functionality in the final product (Morr, 1989, Crowley et al., 2015, Schuck et al., 2016). A powder with poor rehydration characteristics does not only affect consumer perception of the product but also causes challenges at the industrial level in unit operations related to wetting (Gaiani et al., 2007, Hellborg et al., 2012, Richard et al., 2012). Consequently, it is of importance to understand which parameters that affects powder rehydration characteristics.

The rehydration properties of powders (e.g. wetting, dispersiability, and solubility) are affected by several factors, such as powder bulk composition, surface composition of powder particles, particle size, particle morphology, degree of agglomeration, cohesiveness, and powder density (Lillford and Fryer, 1998). Thus, it is a complex process involving many variables. Earlier research has shown that the rate-controlling step in the reconstitution process of whey powders is the wetting (Baldwin and Sanderson, 1973, Gaiani et al., 2007, Ji et al., 2016). Further, other studies have concluded that the wetting of powders is highly affected by the surface composition of the powder particles (Fäldt and Bergenståhl, 1994, Millqvist-Fureby et al., 2001). The wettability of powder, for example, is favoured by hydrophilic molecules on the surface, and a high protein surface coverage reduces the wetting rate compared to lactose dominated particles (Fäldt and Bergenståhl, 1994, Lillford and Fryer, 1998, Silva and O'Mahony, 2017). Further on, the particle morphology is highly dependent on the surface composition of the powder particles (Nijdam and

Langrish, 2006, Nuzzo et al., 2015a). Thus, using microscopical techniques and other detailed methods can contribute with valuable information regarding the particle morphology and surface composition, and this in turn will increase the understanding of the rehydration properties of spray-dried whey protein powders.

Hypothesis

The hypothesis of this thesis is that changes in composition and solution properties and physical state of whey proteins during processing, drying and storage are important for powder functionality. It is also assumed that these changes are reflected in surface rheology properties, aggregation phenomenon and powder particle microstructure.

Aim and Objectives

The overall aim of this doctoral thesis was to gain deeper knowledge regarding the feed properties and how that affects surface composition, particle morphology and rehydration properties of spray-dried whey protein powders.

This overall aim was divided into the following specific objectives:

- Relate surface properties of the feed droplet to the particle morphology of spray-dried whey protein/lactose powder particles (**Paper I, V)**
- Characterize the internal and external distribution of powder constituents in spray-dried whey protein/lactose powders (**Paper II, V**)
- Investigate how the feed composition affect wetting and dissolution of the whey protein/lactose powders (**Paper II, IV, V**)
- Determine the extent of lactosylation in stored whey protein/lactose powders and how it affects the rehydration properties (**Paper III, IV**)
- Evaluate how the degree of protein denaturation and aggregation state of the proteins affects the rehydration properties of whey protein/lactose powders (**Paper III, IV, V**)

The results obtained in this thesis are intended to be used to optimize spray-dried whey powder products with improved rehydration properties.

Background

Whey protein powders

The production of milk proteins is growing rapidly worldwide due to its nutritional and functional properties (IDF, 2019). As milk protein consists of casein and whey, different protein materials can be produced using different manufacturing processes (Oftedal, 2013). Around 80% of the whey powders is produced from liquid whey which is a product from cheese manufacturing, and the rest is derived from casein production (IDF, 2019). The liquid whey consists of 1% proteins, 4% lactose and some minerals. To increase the protein concentration and reduce the amount of lactose and minerals, the whey is subjected to ultrafiltration.

Figure 1.
Examples of whey powder applications (TheNounProject, 2020).

Whey powders are mainly produced by spray drying. There are three primary types of whey protein powders; whey protein concentrate (WPC), whey protein isolate (WPI), and whey protein hydrolysate (WPH). WPC contains fat and lactose, and the protein concentration varies from 30 up to 90%. WPC is produced from liquid whey. WPI are produced through microfiltration followed by ultrafiltration or ion exchange. WPI contains normally at least 90% protein and less fat and lactose than

WPC. WPH consists of whey proteins that have been partly hydrolysed. It is generally accepted that protein hydrolysates containing mostly di- and tripeptides are adsorbed faster than intact proteins (Manninen, 2009). However, some research have reported conflicting results (Farnfield et al., 2008, Cotter and Barr, 2012). Whey powders can be used in many applications due to their functional and nutritional properties, such as in infant formulas, performance products and confectionary goods. Figure 1 displays some of the applications whey powders are used in.

Whey proteins

About 20% of the total protein fraction in milk (5-7 $g \cdot L^{-1}$) consists of whey proteins (WP) (Walstra et al., 2006). The three major whey proteins are β-lactoglobulin (β-lg), α-lactalbumin (α-la), and bovine serum albumin (BSA) (see Table 1). WPs are known for having good essential amino acid balance, high nutritional quality and digestibility (Lollo et al., 2011). WPs are globular proteins where hydrophobic residues normally are found in the core of the protein and the hydrophilic residues are bound outwards (Dill, 1990). The folding of the globular proteins is crucial for their physiological function. α-la works as a coenzyme in the synthesis of lactose. Further, α-la has one strong binding site for calcium (Walstra et al., 2006). The calcium ion is strongly bound and stabilizes the protein conformation. β-lg is the major whey protein. Its solubility and the conformation strongly depend on pH of the solution. In the pH range from 5.2–7.5, β-lg exists as a dimer of two monomeric subunits that are noncovalently bound. Dissociation to the monomer occurs below pH 3.5 and above 7.5. Between pH 3.5 to 5.2, β-lg can form octamers (Walstra et al., 2006, Fox et al., 2015). Above pH 8, aggregation can occur due to formation of intramolecular disulphide bonds (Sawyer, 2003).

Table 1.
Properties of β-lactoglobulin (β-lg), α-la (α-lactalbumin), and Bovine Serum Albumin (BSA) (Walstra et al., 2006).

Whey proteins	Distribution (%)	Molecular weight (Da)	Denaturation temperature (°C)	S-S bridges/molecule
β-lg	55-60	18 320	70	2 + 1 SH
α-la	20-25	14 176	64	4
BSA	5-10	66 267	65	17 + 1 SH

Protein aggregation

Globular proteins are stabilized by several weak bonds and the conformational stability is relatively small (Walstra et al., 2006). Several factors can cause denaturation of globular proteins, such as high temperatures (in general, >70°C), high pH (8-9), and addition of reagents that breaks up H-bonds. Denaturation leads to loss of the proteins' functionality as the protein unfolds. In the unfolded state, the

proteins are more reactive and may undergo covalent bond changes, which then prevent refolding of the protein. When hydrophobic side chains are exposed as a result of protein unfolding, the protein tends to form intramolecular hydrophobic bonds which can lead to precipitation and aggregation, unless the electric charge is high (Walstra et al., 2006). Unfolding and aggregation especially occur at high temperatures. In the production of dairy products, heat treatment is a very common unit operation with the purpose to kill microorganisms and inactive enzymes to produce safe products with increased shelf-life. Heat treatment can also be applied to induce changes to give products specific properties, such as in the production of yoghurt. Further, denatured whey proteins can give a slightly higher viscosity of the product (Carr et al., 2003).

When β-lg unfolds, the protein is prone to aggregate due to exposed free thiol groups. Even though, α-la has a lower denaturation temperature (~64°C at pH 6.7 (Mcguffey et al., 2005)) than β-lg, it is less prone to aggregate due to lack of free thiol groups. The denaturation of α-la is largely reversible at temperatures below 90°C if no other proteins are present (Wijayanti et al., 2014). Caussin et al. (2003) observed that the amount of native α-la was reduced much more when it was heated with other whey proteins, especially with BSA (due to a lower denaturation temperature compared to β-lg, see Table 1). The denaturation of whey proteins depends on several factors, such as pH, protein concentration, ionic strength, calcium activity, and temperature. The pH affects the electrostatic repulsion between the proteins and free calcium ions can form salt bridges which in turn affects the electrostatic repulsion (Walstra et al., 2006).

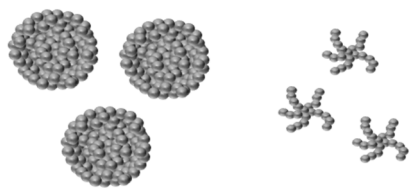

Figure 2.
Schematic overview of whey protein microgels (100 -1000 nm) and fractal aggregates (30-60 nm).

Denaturation and aggregation of whey proteins affect both their functional and nutritional properties. Denatured whey proteins are less soluble than native proteins (Huffman, 1996). Further, proteins that aggregate can become insoluble and precipitate. Powders containing denatured proteins have shown to have poorer wettability, most likely due to the exposure of more hydrophobic groups (Millqvist-Fureby et al., 2001). In this thesis, the effect of protein denaturation and aggregation on rehydration properties were investigated in serum protein/lactose (% w/w) 1/99 and 60/40 powders (**Paper IV**). The systems were heat treated either with or without additional lactose at 70, 75 or 80°C for 15 s. Heat treatment with additional lactose could have an effect on the degree of protein denaturation as well as the lactosylation

of the WPs. The serum protein/lactose (% w/w) 1/99 powder was chosen as it was expected to observe larger effects of the protein denaturation as a large fraction of the bulk protein can be found on the surface (~15%) (Landström et al., 2000). The serum protein/lactose (% w/w) 60/40 system was investigated as it is more similar to commercial powders in relation to the protein content. In addition, a holding time of 15 s was used as that is around the holding time for pasteurization in the dairy industry.

Besides unwanted protein denaturation as an effect of heat treatment, it is possible to tune the conditions (such as pH and salt concentration) to obtain aggregates with different properties. For example, globular proteins are able to form irreversible well-defined particulated aggregates (microgels) when heated in an environment close to their pI or in the presence of salts (Gagnaire et al., 2020). Microgels have been shown to be promising in food delivery systems and to stabilize food-graded emulsions (Destribats et al., 2014). By varying the pH and increase the electrostatic repulsion of the proteins, fractal aggregates can be produced (Kharlamova et al., 2016). Fractal aggregates are branched and are much smaller in size (30-60 nm) compared to microgels (100-1 000 nm) (see Figure 2). Fractal aggregates and microgels from whey proteins could be used to substitute texturing agents in dairy products (Gagnaire et al., 2020, Lesme et al., 2019). Thus, it is of interest to investigate whether they keep their structure after spray drying as it is essential in order to maintain their functional properties. In this thesis, microgels and fractal aggregates were prepared by heating serum protein/lactose (% w/w) 40/60 systems at 85°C for 15 min by alter the pH to 7.3 and 8.6, respectively (**Paper V**). Moreover, the native fraction of serum proteins was varied in the systems to investigate whether that could have an effect on the rehydration properties. It could be expected that native proteins prohibit enhanced wettability compared to denatured and aggregated proteins thereby resulting in an improved wettability of the powders.

Serum protein concentrate

In this thesis, serum protein concentrate (SPC) has been used as a material with addition of lactose in varying ratios. SPC is produced by microfiltration where the caseins are removed (see Figure 3).

Figure 3.
Flow scheme of the production of serum protein concentrate (SPC). Caseins are removed through microfiltration. The protein content is increased in the SPC through ultrafiltration where water, minerals and lactose are removed. The SPC was stored at -18°C until further use.

SPC is a product with low opacity with a pH close to neutral and has a large fraction of native proteins. Compared to cheese whey, SPC is not subjected to as many processing steps that may alter the flavour (Evans et al., 2010). Further, SPC contains lower amounts of fat than WPC, which makes it less prone to lipid oxidation and off-flavour formations. In addition, Punidadas and Rizvi (1998) demonstrated enhanced foaming properties of dialyzed SPC (5%, pH 7.0) compared to products made from cheese whey. The composition of the SPC is displayed in Table 2.

Table 2.
Composition of serum protein concentrate. The compostion varied depending on batch. Three different batches were used in this thesis.

Composition	
Ash (% w/w)	0.6 – 0.8
Dry matter (% w/w)	22 – 30
Protein (% w/w)	18 – 28
- α-lactalbumin	3.2 – 5.2
- β -lactoglobulin	11 – 17
Lactose (% w/w)	1.0 – 2.0
Lipids (% w/w)	~0.1
Phosphorous (% w/w)	0.06 – 0.1
Calcium (bound, % w/w)	0.08 – 0.1
Calcium (free ions, $mmol \cdot L^{-1}$)	4.0 – 5.0
Potassium ($mmol \cdot L^{-1}$)	26 – 46
Sodium ($mmol \cdot L^{-1}$)	4.3 – 18
pH	6.5 – 6.7
Conductivity ($mS \cdot cm^{-1}$)	2.3 – 5.3

Table 3 shows a summary of the experimental setup in **Paper I-IV**. In **Paper V**, the protein/lactose ratio was kept to 40/60 (% w/w) but the ratio of aggregated/native proteins was varied between 1.5 and 32%. Note that "native" proteins are referred to proteins that have undergone no heat treatment prior to spray drying. WPs can be affected by the drying process, however, it has been observed in this thesis that the drying had a limited effect on the native protein fraction (**Paper III**).

Table 3.
The experimental setup in **Paper I–V**.

Paper	Protein/lactose (% w/w)	Dry matter of protein and lactose (% w/w)
I	0.1/99.9, 1/99, 10/90, 40/60, 60/40, 90/10*	17.5
II	0.1/99.9, 1/99, 10/90, 40/60, 60/40, 90/10*	17.5
III	1/99, 60/40, 95/5	22.5
VI	1/99, 60/40	22.5
V	40/60	15

*Marked as SPC/lactose (% w/w) 100/0 in **Paper I**, **II**

Lactose

Lactose is the major carbohydrate in milk and is a disaccharide composed of glucose and galactose. This sugar is unique for milk. Lactose is a reducing sugar that can act as a reducing agent as the sugar can convert to an open-chain structure with an aldehyde group that can react with other components, such as amino acids (Walstra et al., 2006, Van Renterghem and De Block, 1996). This reaction is an early stage in the Maillard reaction. As the Maillard reaction advances, brown coloured furfural compounds are formed (Van Renterghem and De Block, 1996, Le et al., 2011). Lactose exists in both amorphous and crystalline states (α-lactose monohydrate, β-lactose, and α-lactose anhydrate) (Roos, 1995). Upon spray drying, lactose becomes amorphous as the drying time is too short for the lactose to crystallize (Haque and Roos, 2004). As amorphous lactose is hygroscopic, it is very susceptible to moisture uptake during storage (Berlin et al., 1968). Amorphous lactose is in the glassy state as long as the critical water activity (a_w) is below 0.37 (at 25°C) (Roos, 1995). However, the critical a_w and the rate of lactose crystallization depends on temperature and relative humidity. An increase in the relative humidity increases the water adsorption, which in turn increases the plasticization and molecular mobility. Further, as lactose crystallize, water is released, which can cause caking of powders due to solid/liquid-bridging between particles (Roos, 1995, Huppertz and Gazi, 2016). Crystallization of lactose affects the properties of milk powders, such as decreased solubility, loss in emulsifying properties, and reduced flowability (Thomas et al., 2004, McCarthy et al., 2013). As lactose crystallization proceeds, the free fat at the surface increases in spray-dried dairy emulsions, which enhance the development of off-flavours and decreased solubility (Jouppila and H., 1994, McCarthy et al., 2013). Further, lactose crystallization can modify the protein structure as the crystalline lactose destabilizes the hydrogen-bonding between lactose and protein that otherwise stabilizes the proteins to keep their native structure (Thomas et al., 2004). Consequently, it is important to store powders at low temperatures and low relative humidity in order to keep their functional properties.

Spray Drying

During industrial spray drying, the liquid feed is atomized into small droplets in a chamber. The droplets are dried by hot air as they fall in the spray drying chamber. Co-current air flow is normally used for heat sensitive products as the feed temperature is lower than the drying air. Drying from droplet to powder particle is a very rapid process and takes only seconds. Spray drying is a gentle process where the particles are only subjected to a temperature slightly higher than the wet-bulb temperature of the drying air (Masters, 1991). The evaporation of the moisture has a cooling effect, thus, the highest temperature the product is subjected to is the outlet temperature. A schematic overview of the spray dryer used in this thesis can be seen in Figure 4.

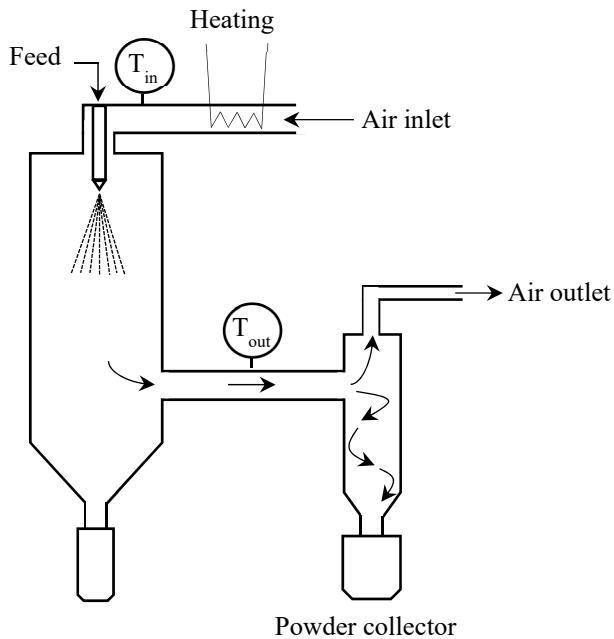

Figure 4.
Schematic overview of a laboratory spray dryer.

The advantages of using a laboratory spray dryer are that it is easy to use, require only small feed volumes, and is not time demanding. However, a very important aspect is that powder particles become much smaller in size (~10µm) compared to industrial spray-dried powder particles (~100µm) and this affects the powder properties. Furthermore, the product recovery is fairly low (~50%) since a large

amount of the particles sticks on the spray drying chamber and there is fraction of fines lost in the exhausting air. Another important aspect in the production of large-scale spray-dried powders is that the material is heat treated and concentrated by evaporation prior to the spray drying as it allows for high viscous fluids and, thus, can handle high concentrations. Also, after the spray drying, the particles go through a fluidized bed for further drying, agglomeration and mixing of the powder particles. Thus, the setup used in this study differs quite much from the industry. However, a laboratory spray dryer is practical to use to produce large powder sets and can contribute to the understanding of how powder properties are affected by different treatments.

During the experimental work in this thesis (**Paper I-V**), a laboratory spray dryer was used to produce the powders. The settings of the spray drying trials are displayed in Table 4.

Table 4.
Settings for the spray drying trials (Mini Spray Dryer B-290, Büchi Labortechnik AG, Flawil Switzerland) used throughout the thesis.

Spray dryer parameter	Settings
Nozzle	Two fluid nozzle
Air flow ($kg \cdot h^{-1}$)	34 (co-current)
Pressurised air ($m^3 \cdot h^{-1}$)	0.8
Inlet temperature (°C)	170 ± 1
Outlet temperature (°C)	80 ± 2
Feed rate ($mL \cdot min^{-1}$)	24

Powder surface formation during spray drying

During spray drying, droplets undergo very fast evaporation and are subjected to both temperature and dimensional changes (Schuck et al., 2016). The lifetime of a droplet in a spray dryer is short and is normally divided into delay time and drying time. The delay time is the time it takes for the droplet to reach the edge of the spray and, thus, the time for the surface-active components to diffuse to the interface of the droplet. The delay time, or the average life-time of 10µm droplet in the wet-zone, has been estimated to be around 0.3 s in a laboratory spray dryer (Fäldt, 1995). The drying time, on the other hand, is much shorter and has been estimated to be around 10-20 ms (Fäldt, 1995, Vehring et al., 2007). During the evaporation, surface active components will enrich at the surface and eventually form a skin. When a critical moisture content is reached, the interface will solidify due to over-saturated conditions in the boundary layer (Masters, 1991). Several models have been proposed to explain the particle formation during spray drying (Charlesworth and Marshall, 1960, Meerdink, 1994, Fäldt and Bergenståhl, 1994, Sadek et al., 2014). According to Charlesworth and Marshall (1960), in absence of surface-active

components and fat, the formation of a solid-like crust on the droplet surface occurs when there is an over-saturation at the surface of the drying droplet and the availability of the solvent is insufficient at the surface. Thus, the composition of the formed crust is expected to be largely dependent on the solubility of the dissolved components. This would lead to a particle surface dominated by the least soluble component. In 1994, it was suggested by Meerdink (1994) that the solutes diffuse towards the centre of the droplet as the solvent diffuses towards the surface, which would lead to a particle surface dominated by the solute having the lowest diffusion coefficient. On the other hand, Fäldt and Bergenståhl (1994) observed that in presence of surface-active components, such as proteins, they will adsorb to the air/water interface of the droplet during spray drying because of their surface activity, and thus will be present on the powder particle surface after drying. Sadek et al. (2014) suggested that, as the saturated droplet surface dries during spray drying, either a ridged or a smooth and flexible skin can be formed. A ridged skin results in either dense, hollow or broken particles due to solidification of the particles, whereas a flexible skin allows inflation and expansion cycles of the droplet as the drying continues, which results in puffed or folded particles. Moreover, the drying temperature and, thus, the evaporation rate has been shown to be of importance related to particle formation and particle morphology (Vehring et al., 2007).

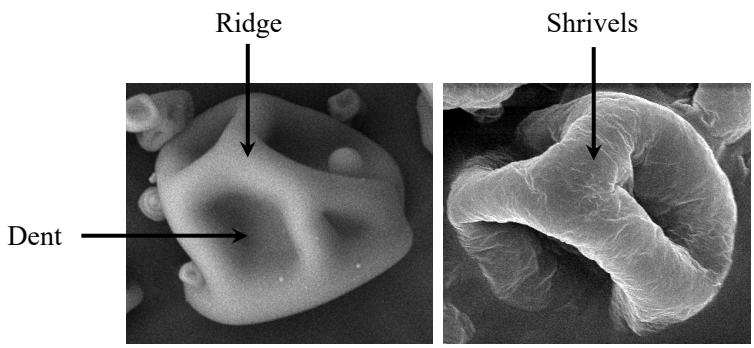

Figure 5.
Descriptives of the particle morphology.

As an example, the drying conditions can cause the particles to collapse or expand, form blow holes, become shelled or to form irregular shaped particles (Vehring et al., 2007). The morphological features of irregular shaped particles can be described as ridges, dents and shrivels. In this thesis, ‘ridges’ is used to describe the continuous elevated crest on the particle, ‘dents’ the indentation in the surface, while ‘shrivels’ are small perpendicular wrinkles on the ridges (see Figure 5). The particles shown in Figure 5 are visualized using scanned electron microscopy (SEM). SEM is a powerful magnification tool that produces high-resolution, three dimensional, and

detailed images which can provide topographical and morphological information. SEM has been used throughout this thesis to characterize the powder particles. However, powder samples are sensitive for the electron beam, and especially powders rich in lactose. Thus, a too high magnification can damage the particles and cause artefacts such as large cracks.

Surface properties of the feed and particle morphology

The interfacial rheology is assumed to play an important role for the surface formation and the morphology of the particles during spray drying of pure protein and non-ionic polymer systems (Elversson and Millqvist-Fureby, 2006, Nuzzo et al., 2015a). The adsorption of proteins to an air/water interface increases the surface pressure as well as the viscoelasticity, which is a measure of the stiffness of the interface. The interfacial properties on an air/water interface can be measured over time by drop tensiometry, and by applying an oscillation this method can supply information about the viscoelastic properties (Loglio et al., 2001). A drawback with the pendant drop technique is that the measurements are performed on a longer time scale compared to the drying time of a droplet in a spray dryer. For that purpose, bubble tensiometry can be used which can measure the surface tension in milliseconds, which has been used in other studies (Porowska et al., 2015, Nuzzo et al., 2015a). However, no concluding remarks related to the surface tension and the particle morphology were made in these studies. However, bubble tensiometry cannot give any information about the viscoelastic properties of the material. In a study performed by Nuzzo et al. (2015a), the authors suggested that the surface elasticity was the most important parameter in relation to the particle morphology. Systems with a low surface elasticity (<10 $mN \cdot m^{-1}$) resulted in particles with a smooth surface, whereas a high surface elasticity (>20 $mN \cdot m^{-1}$) gave rise to more ridged and dented particles. In this thesis, the surface rheological properties were estimated for six serum protein/lactose (% w/w) systems with different ratios (0.1/99.9 to 90/10) using the pendant drop technique (**Paper I**). Since most studies have been conducted on pure or binary protein systems, it is of interest to investigate whether similar observations can be made on more complex and commercial protein systems, such as SPC. This could contribute to further insight in how the surface properties of the droplet are related to the particle morphology. In addition, serum protein/lactose (% w/w) 40/60 systems with different ratios of aggregated and native proteins were investigated to evaluate whether the protein state influences the surface rheology properties (**Paper V**)

Adsorption kinetics of surface-active components, such as proteins, have an important role in spray drying as the time from droplet formation to dried particle is short. The adsorption of these components is controlled by several mechanisms,

such as the diffusivity of the components, hydrodynamic radius, ionic strength, pH, interactions between the components in the feed, and hydrophilicity/hydrophobicity (Tripp et al., 1995, Porowska et al., 2015). In general, a molecule with a higher hydrodynamic radius have a slower diffusion rate. Interestingly, Landström et al. (2000) showed, using fluorescence labelled proteins, that proteins adsorb patch-wise to the interface at low concentration, and that the bulk protein composition of several proteins is reflected in the composition of the protein surface layer. Further, Yang et al. (2020) showed by imaging Langmuir-Blodgett films using atomic force microscopy that native whey proteins had a highly heterogeneous structure at the air/water interface. The proteins formed a dense clustered network which were randomly distributed over the interface. Foerster et al. (2016) analysed the surface composition of cryogenic flash frozen particles and compared with spray-dried particles. Their findings suggested that the protein adsorption seems to develop gradually after atomization of the droplet whereas the distribution of fat at the surface took place during the atomization. This was also suggested in a study performed by Kim et al. (2009) on industrial spray-dried skim milk powders (SMP).

External and internal distribution of powder constituents

The physical structure of dried powder particles is an important factor as the functional properties depend on it (Carić and Kalab, 1987, Sharma et al., 2012). There are several factors that affects properties of spray-dried powders, for example the operation conditions during spray-drying, feed composition, and dry matter content. Table 5 displays some of the factors that affects the properties of spray-dried powders.

Table 5.
Parameters that affect the final properties of spray-dried powders (adapted from Kim et al. (2009)).

Dryer configuration	Feed	Primary powder properties	Secondary powder properties
Inlet temperature	Composition	Moisture content	Sinkability
Outlet temperature	Dry matter	Bulk density	Dispersiability
Type of atomizer	Viscosity	Particle density	Wettability
Type of airflow	pH	Particle morphology	Solubility
Feed rate	Ionic strength	Particle size	Cohesivness
Gas flow rate	Temperature	Colour	Functional properties
	Pre-treatment	Surface composition	Protein solubility
		Internal particle structure	Protein stability
			Flowability

It is usually postulated that the surface properties of spray-dried particles are of importance for powder functionality and affects both the physical and functional properties of the powders, such as wettability, dispersibility, solubility, and flowability. Gaiani et al. (2011) found a correlation between the morphological properties of the particles and the rehydration characteristics. The surface

composition depends on many variables, such as feed composition, degree of heat treatment, and process parameters (Millqvist-Fureby et al., 2001, Anandharamakrishnan et al., 2007, Kim et al., 2009, Fang et al., 2012). Kim et al. (2009) observed a change in the particle morphology of spray-dried SMP as the feed solid content in the feed increased from 10 to 30%. At 10% feed solid content, the particle had deep dents and thick ridges. Further, as the feed solid content increased to 30%, the particles became larger in size, the ridges had become thicker covered with shrivels and the dents had become less deep. Based on their observations, the authors concluded that this finding confirms the rapid crust/skin formation at higher dry matter contents in the feed (Kim et al., 2009). Besides the differences in the particle morphology, the ratio of lactose to protein at the surface increased with feed solids content. A similar observation was made by Millqvist et al. 2001 on dairy emulsions with varying fraction of insoluble proteins. As the fraction of insoluble proteins increased, the ratio of lactose to protein tended to increase on the surface. Fäldt and Bergenståhl (1994) observed that the particle morphology highly depended on the protein/lactose ratio in the feed. Furthermore, by using X-ray photoelectron spectroscopy (XPS), the authors found that spray-dried lactose and protein powders mainly were covered with proteins even at very low protein concentrations. Other studies on dairy powders confirm these findings (Kelly et al., 2015, Nuzzo et al., 2015a). XPS is a powerful quantitative technique to characterize the elemental composition of solid surfaces and has a scanning depth between 2 and 10 nm. The thickness of a protein monolayer at the surface of a powder particle can be expected to between 2 and 5 nm (Fäldt et al., 1993). Furthermore, it was shown in Fäldt et al. (1993) that an underlying layer contributes less to the signal for a spherical particle than for a flat surface as long as the surface layer is thicker than ~8nm. Thus, protein aggregates present just underneath a thin protein layer could therefore contribute to the signal if the native proteins are not distributed in thick layers at the surface (Fäldt, 1995). Due to the limited scanning depth of XPS, the technique does not provide any information about the distribution of the powder constituents in the powder particle matrix (Fäldt et al., 1993).

Several studies have observed phase segregation in spray-dried powder particles containing biopolymers and lactose/maltodextrin (Nuzzo et al., 2015b, Both et al., 2018) The internal distribution of the powder constituents can be expected to have an impact on the functional properties of the powders, such as chemical reactivity, encapsulation efficiency and dissolution properties. Therefore, it is of interest to investigate the phase segregation between proteins and lactose in spray-dried powders. The diffusion rate and the viscosity are expected to play an important role for the internal distribution of powder constituents and, thus, the phase segregation. Several studies have been performed to determine the internal distribution of powder constituents of spray-dried powder particles (Murrieta-Pazos et al., 2012, Nuzzo et al., 2015b, Both, 2019). Murrieta-Pazos et al. (2012) proposed a model for

the surface gradient composition into a depth of 1µm in commercial skim milk powders using XPS connected to Energy Dispersive X-ray. Their findings suggested a model where the protein gradient declines in depth going from the surface into the powder particle whereas the lactose gradient first declines from the surface to increase again moving further into the powder particle. Nuzzo et al. (2015b) investigated the internal distribution of BSA and lactose in single-dried particles using confocal Raman microscopy (CRM). They observed a phase segregation between the protein and lactose up to a BSA concentration of 10% w/w. However, as the BSA concentration increased to 20 % w/w, the outer protein layer seemed to become thinner. On the other hand, using poloxamer instead of BSA, the surface layer became thicker with increasing poloxamer concentration. Both et al. (2018) observed that a slower drying of whey protein/maltodextrin (5/95, % w/w) systems resulted in more extensive phase separation between the two components using CRM. The author hypothesized that the proteins dominate the skin properties and particle morphology at slower drying rates of single-dried droplets.

In this thesis, CRM has been used to investigate the internal distribution of protein and lactose in spray-dried serum protein/lactose (% w/w) 10/90, 40/60 and 60/40 powder particles (**Paper II**). As earlier studies have focused on lower protein-to-lactose ratios, these ratios were chosen to see how the phase segregation between protein and lactose (in depth) were affected by higher protein concentration in the particles, and if that could be related to the rehydration properties of the powders. Figure 6a shows an example of a particle that has been scanned in depth (xz-scan). The white line marks where the depth scan has been performed, going from the upper left to the lower right. Figure 6b shows a CRM image from a depth scan of the powder particle in Figure 6a. As seen, the distribution of protein (green) and lactose (red) can be distinguished. The SEM micrograph in Figure 6c displays a similar particle to the one that has been analysed in Figure 6a-b. The arrows in Figure 6b-c display a similar location that has been analysed. As seen, vacuoles in the particles result in black areas. Further, the intensity of the signal decreases with depth in the particle, which reduces the collection efficiency (Esposito et al., 2016). The advantages of using CRM to localize the powder constituents in powder particles are that the technique does not require any sample preparation and is non-destructive (Heraud et al., 2017). On the other hand, the spatial resolution is around 300 nm which makes it challenging to observe domains smaller than that. Further, as the analyses is quite laborious, only a limited number of particles have been analysed in this thesis.

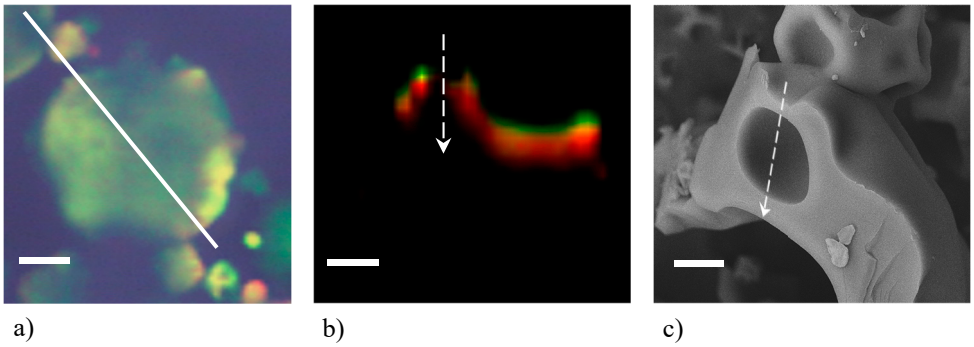

Figure 6.
a) A light microscopy image with an optical overlay showing the particle that has been analysed in b). The white line marks where the depth scan has been performed, going from the upper left to the lower right corner. The green and red colour represent serum protein and lactose, respectively. b) Confocal Raman microscopy (CRM) image of serum protein/lactose (% w/w) 10/90 powder particle. c) A scanned electron micrograph giving an example of a powder particle with similar cross-section to the one in b). The arrows in b) and c) demonstrate an example of a similar location that has been analyzed. The scale bars correspond to 3µm. The particles in b) and c) are not totally in scalar proportions but is an attempt to show how the internal structure gives raise to the CRM image in b).

Lactosylation of whey proteins

Even though spray drying is a gentle process, research has shown that lactosylation of whey proteins can occur during the drying (Guyomarc'h et al., 2000, Norwood et al., 2017, Aalaei et al., 2016). The lactosylation process (or Maillard reaction) is a complex set of chemical reactions where amino groups react with reducing sugars to from various chemical compounds. In whey, lactose reacts with amino acids in proteins, most commonly with lysine. Lysine is an essential amino acid, thus, as a result of lactosylation, the lysine becomes less available as a nutrient for consumers (Friedman, 1996, Rauh et al., 2015). Further, the lactosylation reaction occurs during storage of the powders where temperature and humidity are important factors (Norwood et al., 2017, Aalaei et al., 2016, Pereyra Gonzales et al., 2010). However, lactosylation of proteins can affect the functional properties of the proteins, such as emulsifying and foaming capacity, and the solubility (Thomas et al., 2004). Nacka et al. (1998) and Groubet et al. (1999) observed enhanced emulsifying and foaming properties of lactosylated proteins. However, as the lactosylation continues, the proteins might become less flexible due to an increased number of lactose residues and thus become less effective as emulsifying agents. Nacka et al 1998 observed a higher solubility of proteins substituted with lactose. On the other hand, Stapelfeldt et al. (1997) observed a decreased solubility of milk powders as the hydroxymethylfurfural content (Maillard reaction product) increased. However, lactosylation, which is the first step in the Maillard reaction, may not be the stage responsible for the decrease in the solubility of milk powders.

To evaluate the effect of lactosylation of α-la and β-lg on rehydration properties in this thesis, serum protein/lactose (% w/w) 1/99 and 60/40 powders with varying fractions of native and denatured proteins were investigated (**Paper III, IV**). To induce lactosylation of the proteins, the powders were stored at a_w 0.23, at 30°C for 25 days. An a_w of 0.23 was used in order to avoid recrystallization of the lactose. The serum protein/lactose (% w/w) 1/99 system was chosen as it is expected to observe larger effects in a powder with a high amount of lactose and where a large fraction of the bulk proteins can be found on the surface (~15%) (Landström et al., 2000). The serum protein/lactose (% w/w) 60/40 system was chosen as it more similar in composition to commercial powders.

Rehydration properties

Powder rehydration is essential as the powder needs to be fully dissolved in order to express their functional properties as well as to fulfil the specified chemical composition and nutrient content in the final product (Kinsella, 1984, Crowley et al., 2016). A powder with poor rehydration characteristics does not only affect consumer perception, but also causes challenges at the industrial level in relation to wetting as a unit operation (Schubert et al., 2003, Hellborg et al., 2012, Forny et al., 2011). It is therefore of great importance to understand the parameters that affects powder rehydration characteristics.

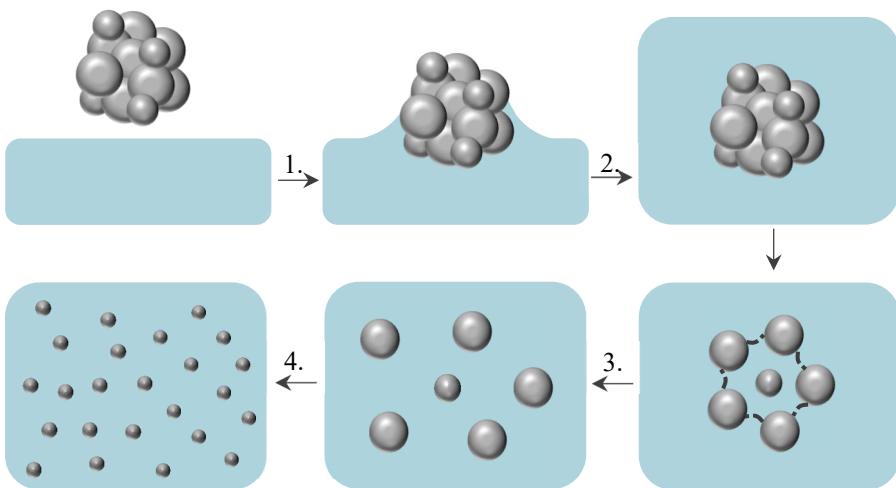

Figure 7.
The rehydration stages of agglomerated whey powder. 1. Wetting, 2. Sinking, 3. Disintegration and 4. Dissolution (Adapted from Forny et al. (2011)).

The rehydration stages of whey powders are usually divided into wettability, sinkability, dispersibility, and solubility (Freudig et al., 1999). Wettability refers to the capacity of the powder particle to absorb water onto the surface, whereas sinkability is the ability of the particle to sink into water. Dispersibility refers to the ability of individual particles to disperse from lumps and agglomerates. The final stage, solubility, corresponds to the separation between molecules of the powder constituents (Freudig et al., 1999). An overview of the different rehydration stages is displayed in Figure 7. It should be noted that swelling of powder particles is another stage that affects the rehydration process, especially for casein powders. However, swelling is not applicable for whey proteins as these globular proteins binds much less water compared to caseins (Kinsella, 1984). The rehydration stages are affected by several factors such as powder composition, particle size, degree of agglomeration, particle density, and chemical composition at the powder particle surface (Lillford and Fryer, 1998, Fäldt and Bergenståhl, 1994, Gaiani et al., 2007). For example, the wetting rate of powders is favoured by small hydrophilic molecules at the surface. A high surface coverage of proteins shows slower wetting rates compared to lactose dominated particle surfaces (Nijdam and Langrish, 2006). In addition, if fat is present in the powders, some of the fat will be present on the particle surface which also has a negative impact on the wetting rate (Fäldt, 1995). Gaiani et al. (2009) observed by using a turbidity sensor that the wettability of WPI powders were poor compared to native phosphocaseinate powders. Further, the turbidity profile showed quite much scattering during the wetting stage, which was suggested to be caused by lump formation. Vos et al. (2016) investigated the water transfer into milk protein concentrate (MPC) powders using Broadband Acoustic Resonance Dissolution Spectroscopy. The results indicated that the water transfer into the MPC powder particles became inhibited as the protein content of the MPC powders increased (Vos et al., 2016). Furthermore, the authors observed that the water transfer into the particles with a high protein content continued after the wetting. On an industrial powder bed scale, wetting is governed by capillary forces. A slow wetting rate may lead to gelling of the liquid front which will have a negative impact of the reconstitution process and cause the formation of lumps. In a study performed by Börjesson et al. (2017), the authors found that a large fraction of air was trapped in the imbibed volume during imbibition of a powder bed. A powder bed refers to the dried powder layer that is formed when adding large amounts of powder to a liquid.

To enhance the rehydration characteristics, powder particles can be agglomerated (Pietsch, 2004). Agglomerates are usually produced using a fluidized bed where a binder is sprayed onto the particles. The binder can be water/steam, whey solution or lecithin solution (Pietsch, 2004). The binder makes the particle sticky, allowing the particles to bind to each other. Consequently, agglomerates consist of several powder particles. Studies have shown that the wettability of whey powders often is

enhanced by agglomeration due to the formation of larger pores which favours water penetration (Pietsch, 2004, Gaiani et al., 2007). However, Ji et al. (2016) observed that agglomeration of whey powders had no beneficial effect on dispersibility as it does not affect the structure of the primary particles. Another way to enhance the wettability of whey powders is to use lecithin. A high coverage of lecithin of the particles results in faster wetting (Ji et al., 2017). However, today more and more consumers demand clean labels with few or no additives which challenges the industry to produce food products with less additives (Asioli et al., 2017). In this thesis, non-agglomerated powders have been characterized.

The rate-controlling step for whey protein powders is the wetting ability (Baldwin and Sanderson, 1973, Gaiani et al., 2007, Ji et al., 2016). When whey powder particles come in contact with water, gelatinous impermeable layer can be formed on the interface between the solids and solution thereby making difficulties for the water to penetrate into the powder matrix (Ji et al., 2016). In this thesis, the wetting of the powders has been estimated by evaluating the spontaneous and forced imbibition, respectively. The spontaneous imbibition was estimated by recording the time for a water droplet to penetrate a powder bed (**Paper II, V**). The technique only requires a small amount of powder and requires very few sample preparations steps. However, it should be noted that as the water penetrates the powder bed, some of the powder will dissolve. The forced imbibition rate of serum protein/lactose (% w/w) powders with a fraction of denatured and aggregated proteins was, in this thesis, evaluated by measuring the change is absorbance (at 600 nm) as a function of time during reconstitution of the powders (**Paper IV,V**). The change in absorbance is caused by the protein aggregates. De Wit and Klarenbeek (1986) used a similar approach to estimate the rehydration properties of protein-rich powders. To measure the change in absorbance, powder is evenly distributed on a water surface, and gentle stirring is applied. The solution is then pumped through a flow-through cuvette, which is placed in a spectrophotometer (at 600 nm). The following equation (1) was used to calculate the forced imbibition rate (v) ($mg \cdot min^{-1}$):

$$v = \frac{m}{t_{Abs2} - t_{Abs\,1}} \qquad (1)$$

where t_{Abs2} is the time (min) when the absorbance stabilized, t_{Abs1} is the time (min) when the absorbance deviated from 0, and m the mass of the powder (mg). The method is further described in **Paper IV.** A strong advantage is that only a small amount of material is needed (0.2% w/w), and it is possible to both get information about wetting and dissolution. This method was chosen because its ability to handle even poorly rehydrating powders compared to other standard methods, such as GEA Niro wettability test and IDF dispersiability and wettability test (Niro, 2005, IDF, 1979). On the other hand, some powders give a very low turbidity due to a low concentration of proteins or that the majority of the proteins is in the native state.

Thus, the technique is most suitable for powders that are turbid after reconstitution, such as heat treated and aggregated proteins. The dissolution rate of serum protein/lactose (% w/w) powders in **Paper II** were estimated using a flow-cell based *in-situ* method developed by Börjesson et al. (2013). The technique is based on immobilization of powder particles/aggregates by compression between two object glasses. The dissolution of the powders was followed using an inverted light microscope. The recording of the flow measurements was performed as described in Börjesson et al. (2013). The calculations of the dissolution rate were based on the following relationship (2):

$$N = \frac{dV}{dt}, V = A \cdot h \qquad (2)$$

where N is the dissolution rate ($m^3 \cdot s^{-1}$), V the volume of the powder bed (m^3), t the time (s), A the area of the powder bed (m^2), and h the height of the flow channel (m). Figure 8 shows a schematic overview of the technique By using this method, the rehydration process can be dynamically monitored starting with a dry powder and then followed in a controlled liquid flow (Börjesson et al., 2013). Another important aspect is that the flow cell allows for investigation of dissolution, separated from wetting and dispersion properties. However, only a limited number of powder aggregates can be investigated at a time with this technique and it is quite laborious. Also, some erosion of the powder bed can occur due to the liquid flow and that can influence the outcome.

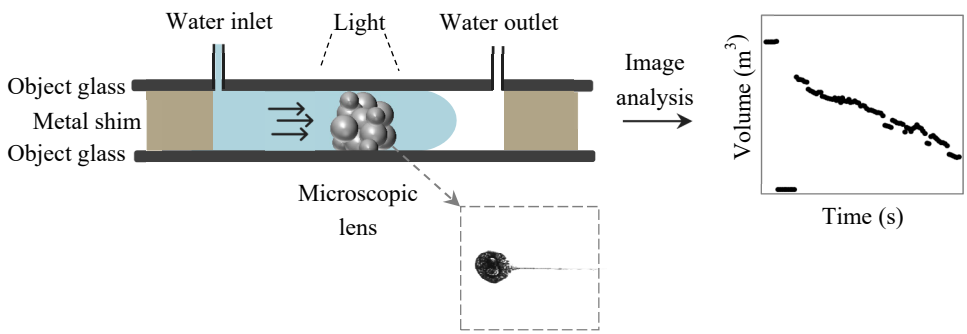

Figure 8.
Schematic overview of the flow cell adapted from Börjesson et al. (2013). The graph shows the output from the image analysis. The volume goes down to zero due to the waterfront passage in the flow cell chamber. The dashed arrow shows the powder after the waterfront has passed. The thickness of the metal shim is 40µm and the distance between the water inlet and outlet is 28 mm. The image is not in scalar proportions. For further description, see **Paper II**.

Surface rheology of the feed and particle morphology

The surface rheology of the droplet of the liquid feed is assumed to be an important parameter to explain the particle morphology of spray-dried particles (Elversson and Millqvist-Fureby, 2006, Nuzzo et al., 2015a). Dynamic surface tension is a suitable method to follow the adsorption of surface-active compounds, such as proteins, to the air/water interface of a droplet. Six different serum protein/lactose (% w/w) systems (17.5% total solids) and five serum protein/lactose 40/60 system (15% total solids) with microgels and fractal aggregates, respectively, with varying amount of native serum proteins were analysed for 2 400 s using the pendant drop technique (**Paper I, V**).

Surface pressure of the feed

The effect of the serum protein/lactose (% w/w) ratio on the surface pressure as a function of time is presented in Figure 9 (**Paper I**).

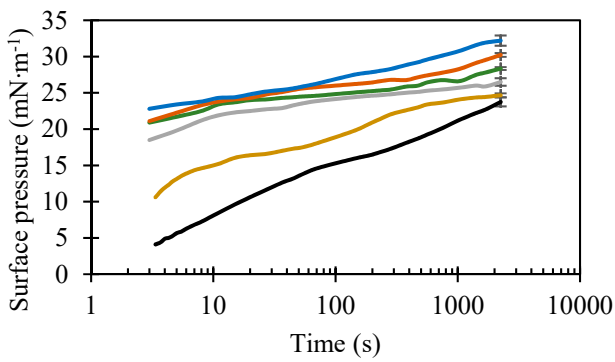

Figure 9.
The surface pressure of serum protein/lactose (% w/w, dry weight) solutions as a function of time. The time to form a droplet (3 s) is added to the time of measurement. All solutions have a total dry matter of lactose and protein of 17.5% (w/w): 0.1/99.9 (black); 1/99 (dark-yellow); 10/90 (grey); 40/60 (green); 60/40 (orange); 100/0 (blue). Standard error of mean is displayed (n=3) (**Paper I**).

As seen, all the systems show a surface activity and an effect on the protein concentration. As shown in **Paper I**, the surface pressure at 0.3 s, which is the estimated life-time of a droplet in the wet-zone in a laboratory spray dryer (Fäldt, 1995), of the serum protein/lactose (% w/w) system 0.1/99.9 (0.018% of proteins) did not deviate from 0 when data from the surface pressure measurements were extrapolated. This implies that less than 50% of a monolayer has adsorbed at the interface (Tripp et al., 1995). On the other hand, the surface pressure at 0.3 s for the serum protein/lactose (% w/w) 1/99 system was estimated to 2.7 $mN \cdot m^{-1}$, which indicates that 50 to 100% of a full protein monolayer has adsorbed to the interface. Formation of monomolecular surface films at the air/water interface can be described by the surface pressure–area isotherm (Larsson, 1994) (Figure 10a). At low protein concentrations, and where the area/molecule ratio is large, the monolayer is considered to be in a gaseous state, characterized by a low surface pressure. As the area/molecule decreases, the monolayer adopts from a gaseous state to a liquid-expanded state, followed by a liquid-condensed state which is characterized by a higher surface pressure. Thus, for the serum protein/lactose (% w/w) 0.1/99.9 system, it is suggested that the surface layer is in the gaseous state and that a non-continuous protein layer has adsorbed to the interface of the system at 0.3 s. As the protein concentration increases in the system, the adsorbed protein at the interface becomes more densely packed. Between the serum protein/ lactose (% w/w) 1/99 (2.7 $mN \cdot m^{-1}$) and 10/90 (17.4 $mN \cdot m^{-1}$) there is a steep increase in the surface pressure at 0.3 s but a moderate increase in the protein surface coverage, which implies that the system has undergone transition from a liquid-expanded to a liquid-condensed phase (see Figure 10b).

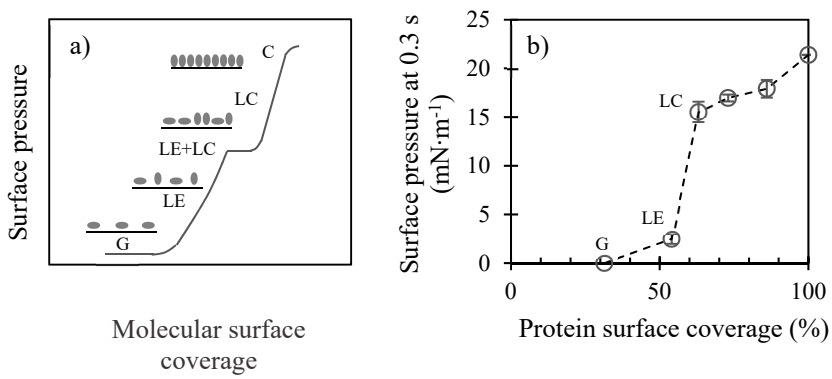

Figure 10.
a) Pressure-area isotherm where the structure of the protein surface layer are indicated; G(gaseous); LE (liquid expanded), LC (liquid condensed), and C (condensed) represents the states ot the two-dimensional surface layer. Modified from Larsson (1994). b) Extrapolated surface pressure of serum protein/lactose solutions at 0.3 s after drop formation of the pendant drop as a function of protein surface coverage of the particles (**Paper I**). Serum protein/lactose (% w/w) system from left to right: 0.1/99.9, 1/99, 10/90, 40/60, 60/40 and 90/10. The standard error of mean is displayed (n=2).

The surface pressure of serum protein/lactose (% w/w) 40/60 systems containing either microgels or fractal aggregates with varying fractions of native proteins are shown in Figure 11. There is no conclusive trend for the microgel system in relation to the amount of native proteins in the solutions. However, the surface pressure at the end of measurements (2 400 s) did not vary to a large extent between the microgel systems (27 to 30 $mN \cdot m^{-1}$). The surface pressure of the fractal aggregate systems was lower compared to the microgel systems. This could be expected as the pH in the systems containing fractal aggregates was 8.6 compared to 7.3 in the microgel system. With increasing pH from the isoelectric point (~5 for β-lg), the electrostatic repulsion between charged groups increases which results in less closely packed proteins at the interface (Meissner at al., 2015). In addition, all the systems investigated had a fraction of native proteins, thus, it could be expected that the interface is dominated by these proteins.

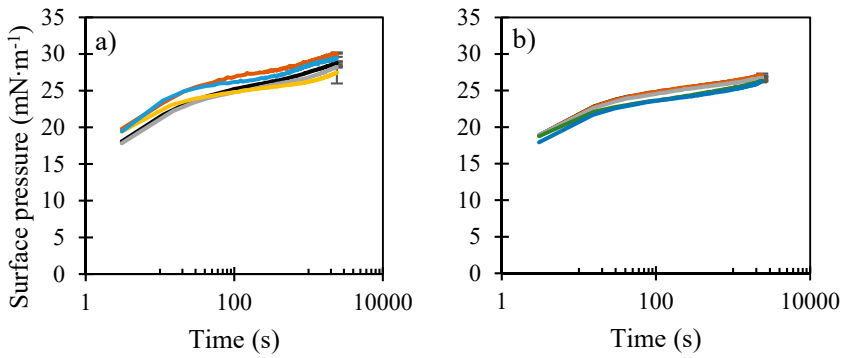

Figure 11.
The surface pressure of serum protein/lactose 40/60 (% w/w, dry weight) solutions with varying fractions of aggregated and native proteins as a function of time; a) microgels and b) fractal aggregates. The vaules are given as the percentage of the total native protein in microgel/fractal aggregate solutions counted on dry matter; 1.5/5 (black); 1.5/4.8 (orange); 4.8/8.0 (grey); 20/20(green); 32/32(blue).The standard error of mean displayed (n=3) (**Paper V**).

Maticorena et al. (2018) observed when investigating WPI systems (80-120 $g \cdot kg^{-1}$, pH 6.5) with different degrees of aggregation (0-80%) that the surface pressure was only slightly affected by the degree of aggregation. The systems with an aggregation degree of 20-80% showed to have a surface pressure of 19 and 21 $mN \cdot m^{-1}$ whereas the native system had a surface pressure of around 23 $mN \cdot m^{-1}$ after 300 s of measurements with the pendant drop technique. This finding indicates that the native protein fraction dominates the adsorption to the interface. On the other hand, Mahmoudi et al. (2011) observed that the surface pressure was slightly lower for native WPI (0.5 $g \cdot L^{-1}$, pH 7.0) system compared to the more aggregated systems (heat treated between 80 to 120°C for 17 to 219 s), ranging from 20 to 23 $mN \cdot m^{-1}$ after 300 s. The system heated at 120°C had approximately 5% residual proteins.

The authors suggested that the non-aggregated proteins in the system dominated the adsorption. Further, when β-lg is heat treated above 60°C at neutral pH, it dissociates from dimers to monomers (Cairoli et al., 1994). Based on this, Mahmoudi et al. (2011) suggested that all residual β-lg molecules were in the monomeric state in the heat-treated systems, and thus, diffused more quickly to the interface compared to the dimers in the native system resulting in the increased surface pressure.

Modulus of elasticity of the feed

By inducing oscillations of the droplet in the pendant drop experiment it is possible to obtain the surface rheological parameters. Adsorption, attachment and conformational changes of surface-active compounds at the droplet surface, as well as lateral interactions between the molecules at the surface, influence the droplet surface rheology.

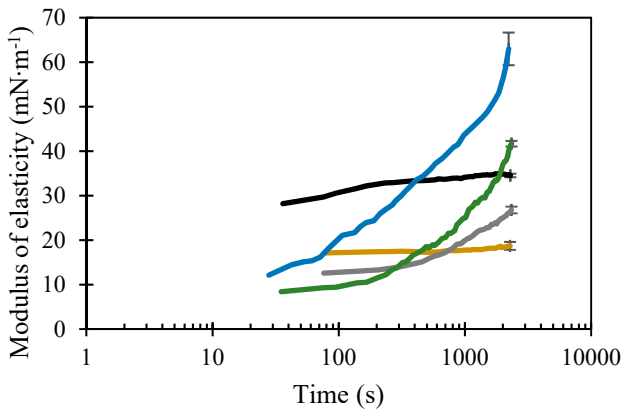

Figure 12.
Modulus of elasticity of serum protein/lactose (% w/w, dry weight) solutions as a function of time. The dry matter content of lactose and protein was kept constant to 17.5% (w/w) in all solutions. 0.1/99.9 (black), 1/99 (dark yellow), 10/90 (grey), 40/60 (green), and 90/10 (blue). Standard error of mean is displayed (n=3) (**Paper I**).

The modulus of elasticity of serum protein/lactose (% w/w) systems are shown in Figure 12 (**Paper I**). The modulus of elasticity increases with increasing protein concentration with an exception for the system 0.1/99.9. This deviation is discussed in **Paper I**. As can be seen in Figure 12, the modulus of elasticity at the beginning of the measurements become lower with increasing serum protein concentration. At higher protein concentrations, a more complex surface is expected to be formed

including the protein diversity of the SPC. Such a surface layer is expected to display a lower surface elasticity, particularly if the system is not fully equilibrated. As the SPC is a chemically complex system, heterogeneities in the adsorbed layer (lateral phase separation) may be expected which is expected to cause a reduction in the modulus of elasticity.

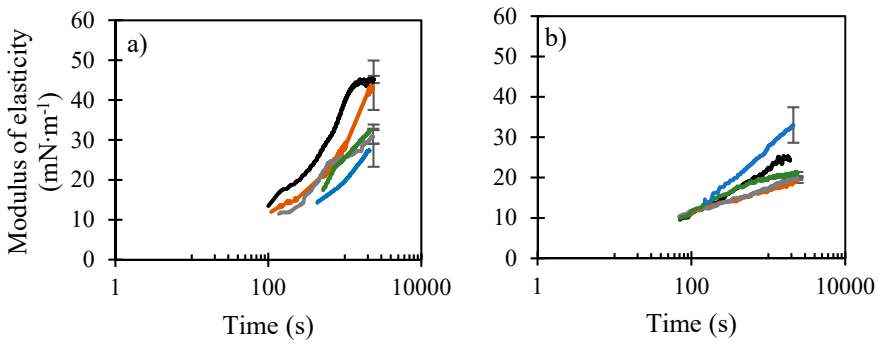

Figure 13.
The modulus of elasticity of serum protein/lactose 40/60 (% w/w, dry weight) solutions with varying fractions of aggregated and native proteins as a function of time; a) microgels and b) fractal aggregates. The values are given as the percentage of the total amount of native protein in microgel/fractal aggregate solutions counted on wet matter (6% protein); 0.3/1.0 (black); 0.3/0.9 (orange); 0.9/1.5 (grey); 3.7/3.8(green); 6.0/6.0(blue).The standard error of mean is displayed (n=2).

Figure 13 shows the modulus of elasticity measurements of microgels and fractal aggregates as a function of time (**Paper V**).As can be seen for the microgels system (Figure 13a), the modulus of elasticity is highest (~45 mN·m^{-1} at 2 400 s) for the systems with the lowest concentration of native proteins. As the native protein fraction increases to 60% in the system, the modulus of elasticity decreases to around 30 mN·m^{-1}. For the fractal aggregate systems, the opposite is observed (Figure 13b). As the native protein fraction increases in the powders, the modulus of elasticity increases from 20 to 36 mN·m^{-1}. Overall, microgels contribute to a stiffer interface compared to fractal aggregates. Similar findings have been observed in a study by Mahmoudi et al. (2011) where larger aggregates formed solid-like layers whereas system with a high amount of native proteins and fractal aggregates formed more fluidlike layers. The modulus of elasticity of an adsorbed protein layer depends on the ability of the protein molecules to rearrange upon a change in surface area. For example, flexible random coil proteins have the ability to rearrange rather quickly, hence, they exhibit low modulus of elasticity whereas globular proteins being slow in rearrangement display much higher modulus of elasticity (Narsimhan, 2016).

Particle morphology related to surface rheology of the feed

The surface rheology of the interface of the droplet is assumed to influence the particle morphology of the dried particle. Large and less flexible molecules that have adsorbed at the interface generate a stiff surface layer, which induces a high modulus of elasticity. Further, the concentration of surface-active molecules adsorbed at the interface, such as proteins, influences both the surface pressure and the modulus of elasticity. An elastic interface would result in a film that does not easily break or contract as the droplet shrinks resulting in dented and ridged particles. On the other hand, an interface with poor elasticity and low surface pressure is expected to contract as the droplet shrinks during drying resulting in smooth particles. The morphology of the powder particles in this thesis was examined using SEM.

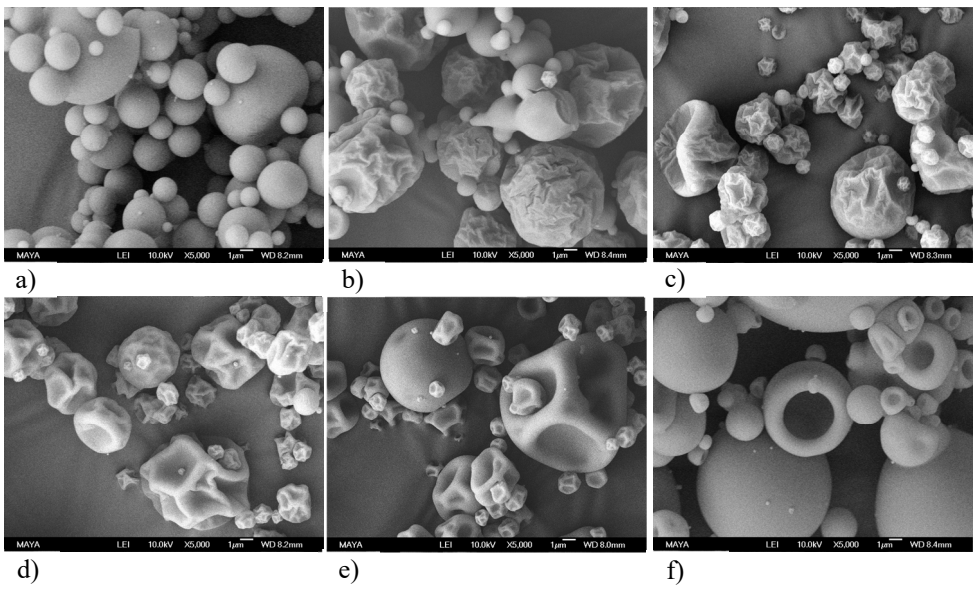

Figure 14.
Scanned electron micrographs of spray-dried serum protein/lactose particles (% w/w, dry weight); a) 0.1/99.9, b)1/99, c) 10/90, d) 40/60, e) 60/40 and f) 90/10. The scale bars correspond to 1µm. Magnification 5 000 x (**Paper I, II**).

Figure 14 shows SEM micrographs of SPC powder particles with varying ratios of serum protein and lactose (**Paper I, II**). As seen, the particle morphology depends on the protein concentration in the powders. The particle morphology of the serum protein/lactose (% w/w) 0.1/99.9 powder has totally smooth particles (Figure 14a).

As the protein concentration increases to 1% (w/w), the particles become covered with a high frequency of dents and thin ridges (Figure 14b). Further, the smaller particles (<3µm) are either smooth or partially dented. At protein concentrations ≥10% (w/w), the frequency of dents and ridges decreases but they tend to become deeper and thicker, respectively (Figure 14c-e). In the powder with the highest serum protein concentration of 90% (w/w), the particles display a single or a few dents (Figure 14f). Similar morphologies have been observed previously in WP/lactose powders (Fäldt and Bergenståhl, 1994, Nuzzo et al., 2015a).

Based on these observations in serum protein/lactose (% w/w) systems, it is suggested that the surface pressure plays an important role for the surface deformation, particularly at low protein concentrations (<1% w/w) where the area/molecule is large. As the protein concentration increases to 1% (w/w), it is assumed that the adsorbed protein layer moves from gaseous state towards a liquid-expanded state. As the protein concentration increases to ≥10 % (w/w), the modulus of elasticity seems to be the most prominent surface property while explaining the particle morphology (Figure 15). It is assumed that the adsorbed monolayer goes from liquid-expanded to liquid-condensed state as the interface becomes more densely packed with proteins. As a result, the modulus of elasticity increases, and the particles become more dented and ridged.

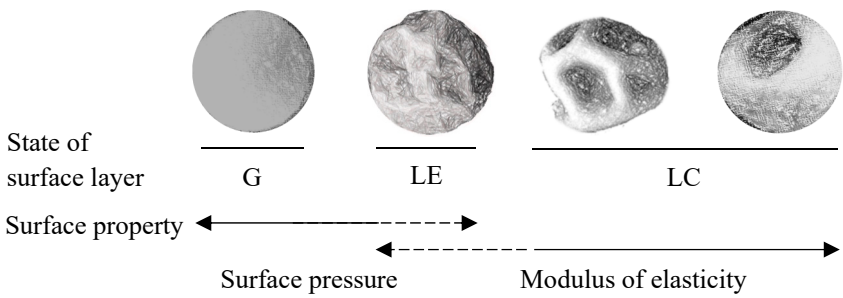

Figure 15.
Schematic overview of the particle morphology, and how it is related to the surface properties of the feed. G (gaseous), LE(liquid expanded), and LC (liquid condensed) represents the two-dimensional surface layer (**Paper I**).

One interesting question is how the particle morphology is affected by the aggregation state of the serum proteins (**Paper V**). Serum protein/lactose (% w/w) 40/60 powders containing varying fractions of native and aggregated proteins (microgels and fractal aggregates) are shown in Figure 16. Overall, the microstructure of the particles is fairly similar where the particles are covered with dents and ridges. Figure 16f shows a native serum protein/lactose (% w/w) 40/60 powder. As seen, the particles are covered by 10 to 20 dents and 0.5 to 1.5µm thick ridges. Further, the small particles (<3µm) is very similar in appearance compared to the larger ones (>5µm).

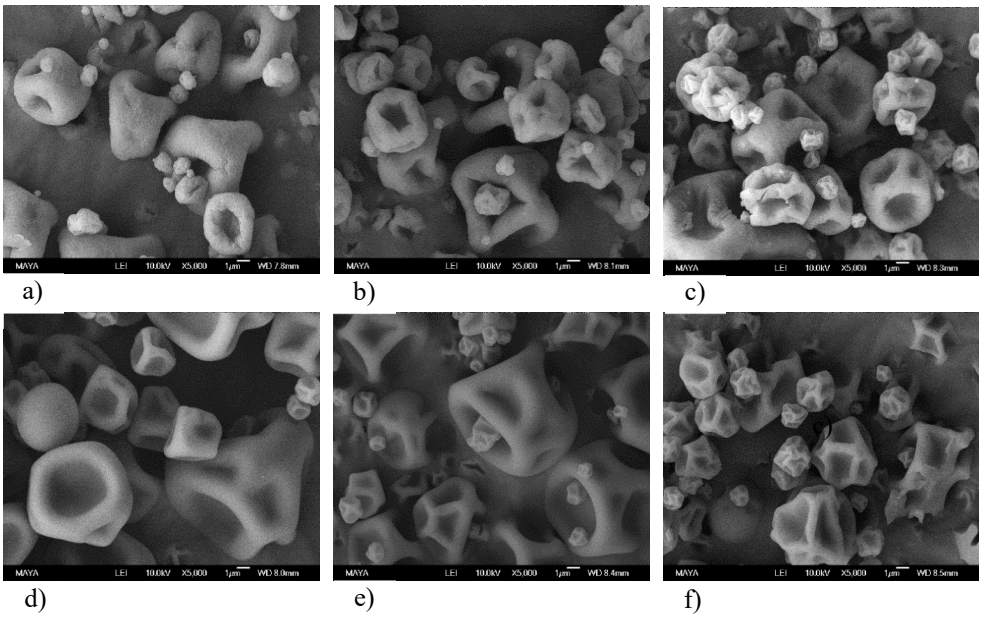

Figure 16.
Particle morphology of aggregated serum protein/lactose 40/60 (% w/w) powders with varying ratios of native proteins. a) Microgels (1.5% native proteins), b) microgels (11% native proteins), c) microgels (20% native proteins), d) fractal aggregates (5% native proteins), e) fractal aggregates (13% native proteins), and f) non-aggregated (100% native proteins) (**Paper V**). The scale bars correpsonds to 10μm. Magnification 2 000 x.

However, comparing the native powder with microgel powders (Figure 16a-c), some differences can be distinguished. The powders with a high fraction of microgels have fewer dents and thicker ridges compared to the powder with only native proteins. Furthermore, the ridges are covered by shrivels, which are not seen in the native serum protein/lactose powder (Figure 16f). The shrivels are expected to be caused by the protein aggregates found at or just underneath the particle surface as they tend to decline in number as the native protein fraction increases in the powders. Also, the smaller particles (<3μm) are folded in a different way where ridges and dents cannot be differentiated to the same extent as for the larger particles. The observed differences could be caused by a faster drying rate (Vehring et al., 2007). The time for native proteins to diffuse and adsorb to the interface of small droplets before a skin has formed is shorter compared to larger droplets. Thus, it is suggested that the smaller particles have more aggregated proteins at or just underneath the surface compared to the larger particles. Further, the size of the aggregates (~250 nm) is relatively large compared to the size of the small particles. The morphology of the fractal aggregate particles is very similar to the native serum protein/lactose particles (Figure 16f), but it is possible to distinguish some shrivels on the particle surface for the powder with a high fraction of fractal aggregates (Figure 16d-e).

Connecting these observations to the modulus of elasticity, the microgel systems with a low fraction of native proteins (1.5%) displayed a high modulus of elasticity (42–45 $mN \cdot m^{-1}$), which resulted in powder particles with a low frequency of dents as well as thick and shrivelled ridges. As the native protein fraction increased in the microgel system, the modulus of elasticity decreased, and the particles became covered with a higher frequency of dents and thinner ridges. A similar observation was made in the native serum protein/lactose (% w/w) system where a high modulus of elasticity resulted in particles with few dents and thick ridges (Figure 15). The modulus of elasticity and the particle morphology (Figure 16d-e) of the fractal aggregate systems (19-31 $mN \cdot m^{-1}$) was lower compared to the microgel system and more similar to the native serum/protein (% w/w) 40/60 system. Thus, the type of aggregate contributes differently to the stiffness of the interface. A system with a high fraction of microgels results in a stiffer interface compared to fractal aggregates, which is most probably caused by their size and low flexibility.

External and internal distribution of powder constituents

The external distribution of proteins at the particle surface was estimated using XPS (**Paper I, V**). SEM was used to characterize the external and internal particle morphology (**Paper IV, V**). The internal distribution of powder constituents was mapped by scanning powder particles in depth (xz-scan) and in the horizontal plane (xy-scan) using CRM (**Paper II**).

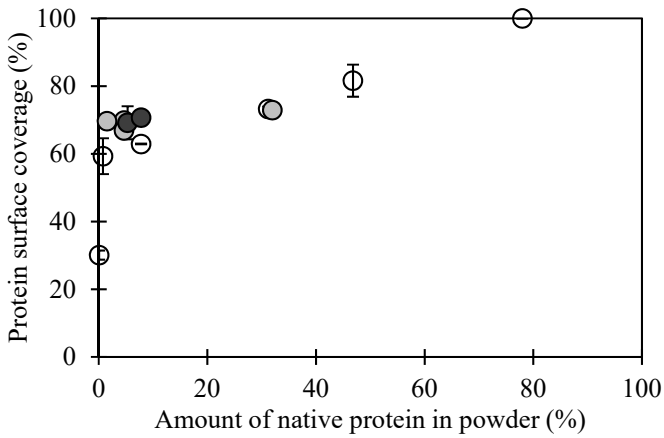

Figure 17.
The protein surface coverage of serum protein/lactose (% w/w) powders as a function of the native amount of proteins in the powders. Data from **Paper I** (white), and **Paper V** (microgel powders - medium-grey and fractal aggregate powders - dark-grey).

The protein surface coverage of spray-dried serum protein/lactose (% w/w) powder particles are displayed in Figure 17. The protein surface coverage of the particles increases with bulk protein concentration in the powders (circles with no filling in Figure 17). At low protein concentrations (≤1%), the protein surface coverage shows a steep increase from 31 to 54% and thereafter a linear increase between 1-90% (w/w) of serum proteins. These findings have been observed in previous studies on dairy powders (Fang, Wang, & Bhandari, 2013; Kelly et al., 2015; Nuzzo, Millqvist-Fureby, et al., 2015). The native serum protein/lactose (% w/w) 40/60

powders show good reproducibility comparing the protein surface coverage from two different batches of SPC (**Paper I, Paper V**). As shown in **Paper V**, the protein surface coverage of aggregated (microgels and fractal aggregates) serum protein/lactose (% w/w) 40/60 powders with varying amounts of native proteins (1.5-5%, dry weight) was between 67 and 70%, and thus similar to the 40/60 powder with 32% native proteins (72%). However, the amount of native (soluble) proteins differed considerably between these powders, thus, the protein surface coverage was recalculated based on the total amount of soluble proteins in the powder by using the linear relationship between the protein surface coverage and protein concentration for the serum protein/lactose (% w/w) powders 1/99 to 90/10 from **Paper I**. It was postulated that the protein aggregates were more less inert relative to the surface as it could be expected that a large fraction of the aggregates is insoluble and that the remaining fraction of soluble aggregates have a slow diffusion to the interface due to their hydrodynamic radius. The result of the recalculation on the soluble protein fraction (1.5-5%) is displayed in Figure 17 (medium-grey and dark-grey circles). As seen, the protein surface coverage is 10-15% higher compared to the powders in **Paper I** that have a similar soluble protein fraction. This implies that the protein aggregates are not fully inert relative to the surface as the protein is 'overrepresented' at the particle surface in these systems relative to the soluble protein fraction.

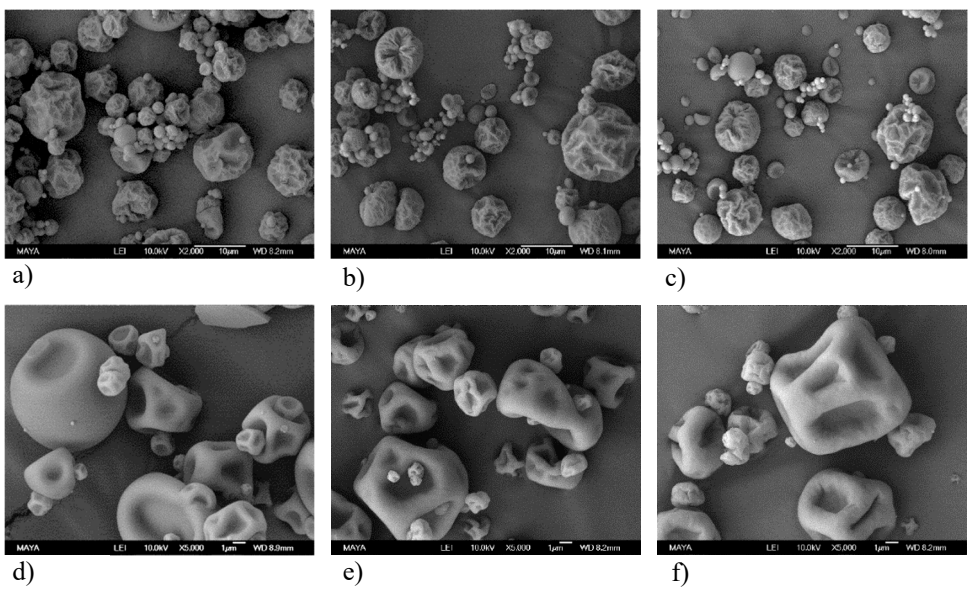

Figure 18.
Scanned electron micrographs of serum protein/lactose (% w/w, dry weight) powders. The values in brackets display the total fraction of native α-la and β-lg. a) 1/99 (100%), b) 1/99 (67%), c) 1/99 (51%), d) 60/40 (99%), e) 60/40 (69%), and f) 60/40 (46%). Scale bars correspond to 10μm in a) to-c) and 1 μ. I d) to f). Magnification 2 000x (a-c) and 5 000x (d-f) (**Paper III, IV**).

However, protein aggregates located just underneath a thin native protein surface layer (<8 nm) can contribute to the elemental composition at the surface. Therefore, the protein aggregates present at the surface could be somewhat lower than 10-15%.

Protein aggregates are normally formed as a consequence of heat treatment as globular proteins are rather heat sensitive. A common processing step in the production of whey powders is heat treatment of the feed prior to spray drying. In this thesis, the appearance of the particles after heat treatment at 70, 75 and 80°C for 15 s was evaluated in serum protein/lactose (% w/w) 1/99 and 60/40 (**Paper III, IV**). The 1/99 system was chosen as a large fraction of the bulk protein will be present at the particle surface (~15%) (Landström, 2000) whereas serum protein/lactose (% w/w) 60/40 is more similar to commercial powders. Figure 18 shows some examples of the powder particle morphology for these systems. Even though a large fraction of the native proteins is denatured (~50%) in the powders, only small changes could be observed in the particle morphology. For the serum protein/lactose (% w/w) 1/99 powders, the size of the smooth and spherical particles tends to become larger as the amount of native serum proteins decreases in the powders. A possible explanation for this could be that the time for the protein to diffuse to the surface of a smaller droplet during spray drying is limited due to a faster drying time compared to larger droplets. In addition, as a consequence of increasing heating temperature, larger fractions of the proteins could be expected to have become insoluble. Further, it could be expected that soluble aggregates diffuse slower to the interface compared to native proteins due to a larger hydrodynamic radius. In the serum protein/lactose (% w/w) 60/40 powders, the smaller particles (<3µm) tend to change in appearance as the native fraction of proteins decreases. For the larger particles (>3µm), the number of dents is slightly reduced, and the ridges tend to become thicker and covered with a few shrivels. This was also seen for the serum protein/lactose (% w/w) 40/60 particles where a large fraction of the protein was aggregated (Figure 16).

One interesting question is, thus, how the interior of the particles looks like. SEM micrographs revealed that protein aggregates could be distinguished in the interior of the powder particles (Figure 19a-c) (**Paper IV, V**). The diameter (Z-average) of the microgels was estimated to be approximately 250-280 nm using dynamic light scattering (DLS) which is approximately the same size as the spherical objects in the SEM micrographs (**Paper V**).

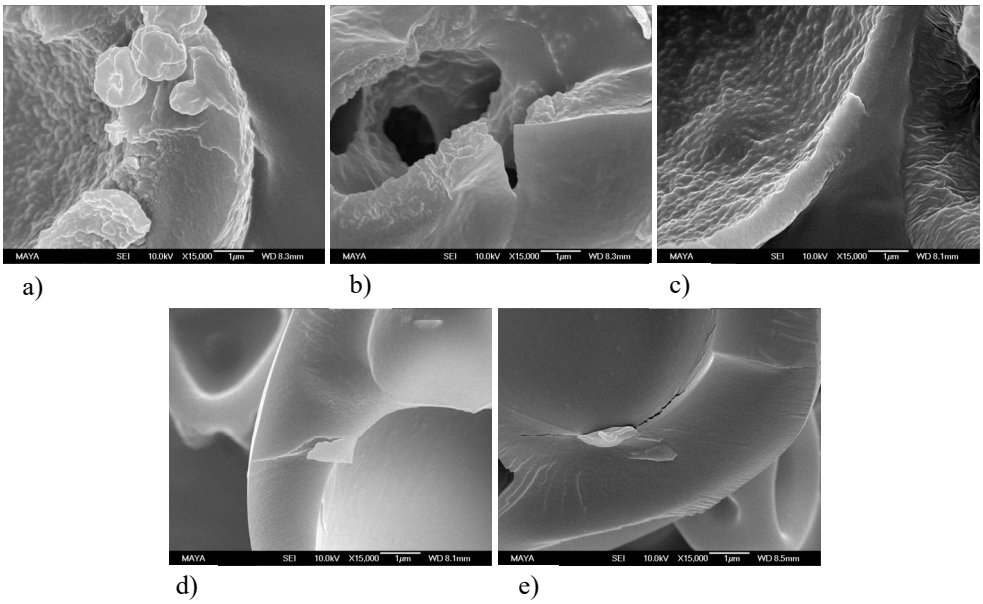

Figure 19.
Cross-section of spray-dried serum protein/lactose (% w/w, dry weight) powder particles with varying fractions of aggregated and native proteins (**Paper IV, V**). The values in brackets are the total amount of native proteins in the powders. a) to c) display microgels and d) fractal aggregates. a) 60/40 (2.3%) b) 60/40 (6%), c) 40/60 (1.5%), d) 40/60 (5%), and 60/40 (32%).

In addition, cryo-TEM images revealed that the microgels were dense and spherical with a size close to the estimations from the DLS measurements (Figure 20a). Further, as seen in Figure 19b, the particle surface is much smoother in appearance compared to the vacuole. The internal structure at the vacuole are very rough whereas the particle surface is smoother in appearance and seems to be covered with a thin layer encapsulating the rough structure. Based on these observations, it is suggested that this thin layer could be a result of native proteins that have adsorbed to the surface during the drying. This is also supported by looking on the cross-section of the particles. The cross-section of the particles with protein aggregates (Figure 19a-c) is rougher in appearance compared to the smooth cross-section of the powder particle with only native proteins (Figure 19e). A smooth cross-section was also observed for fractal aggregate powders containing a low amount of native proteins (Figure 19d). In contrast to the microgels, the fractal aggregates are much smaller in size. A cryo-TEM image of the fractal aggregates after spray-drying and reconstitution is shown in Figure 20b. As seen, there is a large fraction of small aggregates with a size around 10-30 nm as well as clusters with a size around 100 nm. This was also confirmed using DLS. Hence, a large amount of the fractal aggregates is too small to be visually seen in the SEM micrographs.

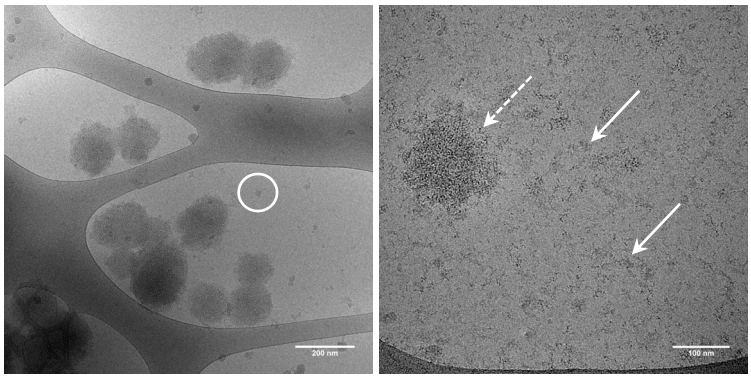

Figure 20.
Cryo-transmission electron micrographs of microgels (a) and fractal aggregates (b) after spray drying and reconstitution. Scale bars correspond to 200μm (a) and 100μm (b). Magnification 40 000x in a) and 80 000x in b) (**Paper V**). The circle shows an example of contamination during the sample preparation with ethane. The dashed arrow marks a cluster aggrgeate and the solid arrows show examples of fractal agrgegates.

The adsorption of surface-active components depends on several parameters such as hydrodynamic radius, diffusion coefficient, interactions between other components in the feed, pH and ionic strength (Porowska et al., 2015). As seen in Figure 9, the surface pressure tends to be higher in systems with a higher fraction of native proteins. Thus, this implies that native proteins adsorb to the interface to a larger extent compared to the denatured/aggregated proteins due to a higher surface activity as well as smaller hydrodynamic radius and hydrophobicity/hydrophilicity. Thus, a higher fraction of protein aggregates could then be expected to be found in the interior of the powder particle than at the powder particle surface. That could explain that the particle morphology on a microscale is rather similar independent of the degree of protein aggregation and denaturation, whereas the nanostructure (shrivels on the ridges) could be caused by protein aggregates situated at or just underneath the particle surface.

As shown in Figure 17, the protein surface coverage increased linearly between serum protein/lactose (% w/w) powders 1/99 to 90/10 (**Paper I**). Hence, it could be assumed that the protein layer at the interface become thicker with increasing native protein content in the powders. The internal distribution of powder constituents in serum protein/lactose (% w/w) powders 10/90, 40/60 and 60/40 are displayed in Figure 21 (**Paper II**). At a serum protein/lactose (% w/w) ratio of 10/90 (Figure 21a), a protein enriched domain (green) at the surface with an approximate thickness of 1μm is distinguishable. The protein rich domain is followed by a lactose-rich domain (red), and a phase segregation between the components is thus observed. As the serum protein concentration increases to 40% (w/w) (Figure 21b), the system becomes more heterogenous and it seems that a lactose-rich domain is found in the vicinity of the particle surface.

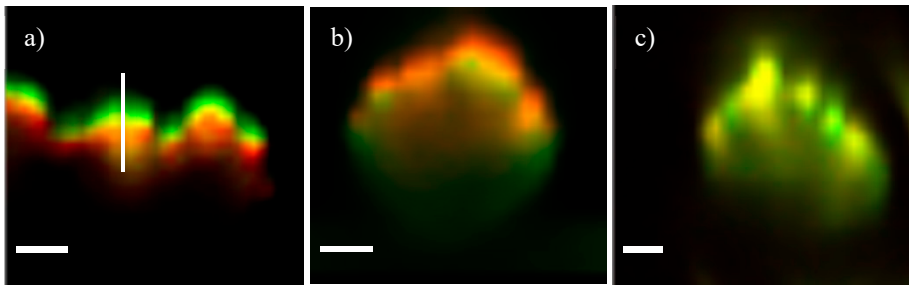

Figure 21.
Confocal Raman images of serum protein/lactose (% w/w) powder particles. a) 10/90, b) 40/60 and c) 60/40. The scale bars correspond to 3µm. Green and red represent serum protein and lactose, respectively. The vertical line gives an example of an area that has been analyzed to obtain the signal from respective species. The relative signal at 0.5 can then be used to obtain the thickness of each domain. This is further discussed in **Paper II**.

A similar observation was made for the particles with a serum protein concentration of 60% (w/w) where the distribution is more heterogenous. For more CRM images, see **Paper II**.

The spatial resolution of CRM is around 300 nm, thus, if the protein domains become smaller in size as the protein concentration increases, the technique will not be able to differentiate them. However, similar observations have been made in studies using WP/lactose systems (Nuzzo et al., 2015c). At protein concentrations ≥10% (w/w), the authors observed that the protein-rich domain tended to become thinner. To estimate the composition and the layer thickness in the vicinity of the particle surfaces in the CRM images, the location of the relative signal intensity reaching a value of 0.5 can be used. The relative intensity refers to the maximum of the intensity of respective species. The analysis was performed in a vertical direction and the white line in Figure 21a gives an example of an analysed location. Several particles with different morphologies (hollow as well as dented and ridged) were investigated for each serum protein/lactose (% w/w) powder. This is further described and discussed in **Paper II**. Figure 22 shows a summary of the surface layer thickness at several location of the powder particles as a function of the bulk protein concentration in the powders (**Paper II**). Here, it is clearly visualized that the protein-rich domain in the vicinity of the surface tends to become thinner with increasing protein concentration. However, the XPS revealed that the protein surface coverage of serum protein/lactose (% w/w) 40/60 and 60/40 was 72 and 86%, respectively. Thus, the results from the CRM is interpreted as that the protein-rich domain in the vicinity of the surface becomes thinner than the resolution limit of this technique (~300nm) when the relative signal of lactose appears before the relative signal of serum protein. A possible explanation for this observation could be that the viscosity of the droplet of the feed increases during the drying, and gets more pronounced as the protein content increases in the feed, thereby slowing down

the diffusion of the globular proteins and, thus, resulting in smaller and less segregated phases (Porowska et al., 2015). Further, it has been observed that the particle morphology is quite diverse within the same powder sample which could be an effect of the drying time (Figure 14). Hollow particles could be formed if the surface of the droplet becomes ridged very quickly (Vehring, 2008). Further, the drying time for smaller particles is shorter than for larger ones, thus, it could be expected that less proteins have adsorbed to the surface causing the variation in the protein layer thickness in the vicinity of the powder particle.

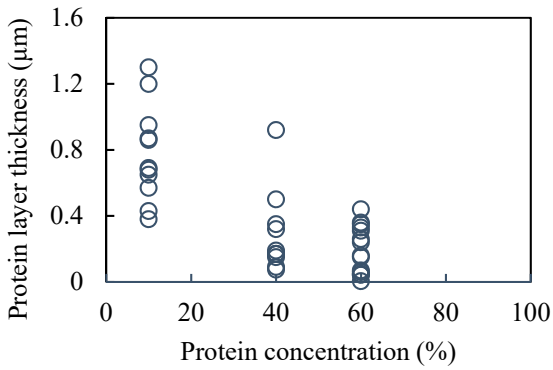

Figure 22.
Thickness of serum protein enriched domain in the vicinity of the surface of serum protein/lactose (% w/w) powder particles 10/90, 40/60 and 60/40 (% w/w) analysed with confocal Raman microscopy (**Paper II**).

Another type of particles observed in this thesis is large hollow particles with very thin walls. These types of particles can be formed if the surface becomes ridged very fast (Vehring et al., 2007). These different types of particles could cause variation in the thickness of the protein layer in the vicinity of the powder particle as well. As an example, Both (2019) observed that the morphology differed depending on the particle size of spray-dried WP/maltodextrin solutions. Larger particles where ridged and dented whereas smaller particles where hollow and smooth (Both, 2019). The authors explained this phenomenon as a result of a faster locking point of the smaller droplets, due to a faster heat and mass transfer than for the larger droplets. In addition, the author concluded that phase separation is unlikely to occur as drying time is very short during spray drying. Nevertheless, in this thesis, the results have indicated that at protein/lactose concentration ≤10/90 (% w/w) phase separation does occur, however, it tends to become less pronounced as the protein concentration increases in the powders.

Rehydration properties of spray-dried SPC powders

The rehydration properties of spray-dried powders are expected to depend on the surface composition of the powder particles. Powders with a more hydrophilic surface is expected to show a faster wetting for example. The powders that were investigated had a volume-weighted diameter of 10 to 18µm, and the moisture content in the powders varied between 1 and 4%.

Impact of protein/lactose ratio in powders

The rehydration properties of spray-dried SPC powders with varying ratios of serum protein and lactose were evaluated using a flow-cell based method. A powder bed was fixated between two object glasses, and the forced imbibition was measured by applying a controlled liquid flow. For more details, see **Paper II**. Figure 23a shows the dissolution rate of the powders.

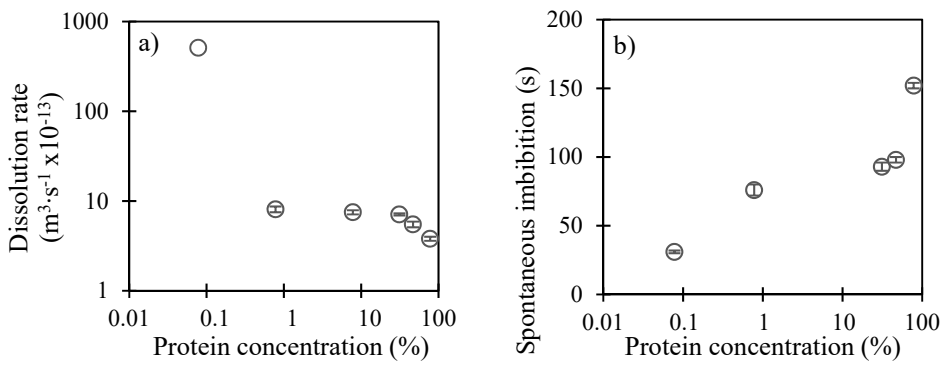

Figure 23.
Dissolution rate ($m^3 \cdot s^{-1}$) (a) and spontaneous imbibition (s) (b) as a function of protein concentration in serum protein/lactose (% w/w) powders (**Paper II**). Standard error of mean is displayed (a n=15 to 39 and b. n=6). The measurements are repeated on two powders with the same composition.

The dissolution rate decreases as the protein concentration increased in the powders (Figure 23a). The powder with 0.1 % w/w protein dissolved immediately as the waterfront passed the powder bed in the flow cell. Surprisingly, as the protein concentration increased to 1 % w/w, the dissolution rate decreased almost by two orders of magnitudes, but the change was very limited with further increase in protein concentration. This might be explained by the protein surface coverage of the powder particles (as shown in Figure 17). At the lowest protein concentration of 0.1 % (w/w), the protein surface coverage was estimated to 31% using XPS. As the protein concentration increased to 1% (w/w), the protein surface coverage increased to 54%. As discussed earlier, it is suggested that the proteins adsorb patch-wise at low protein concentrations (<1% w/w), and as the protein concentration increases to 1% (w/w), the proteins adsorb in a thin and evenly distributed layer. As the protein concentration increases further in the powders, the adsorbed proteins result in a continuous and dense surface layer. The reduction of hydrophilic lactose at the surface of the powder particle, as a consequence of the increasing amount of protein at the surface, may reduce the water transfer into the particle, thereby causing a slower dissolution rate. Vos et al. (2016) observed that the water transfer into MPC powder particles became inhibited as the protein concentration increased in the powders resulting in slower wetting. As the protein surface coverage increases, protein-protein interaction may be promoted, thus hindering the water to penetrate the particle, and thereby slow down the imbibition (Fyfe et al., 2011). Further, a rapid dissolution of the protein from the surface may also lead to an increased viscosity and gel formation within the inter-particle space.

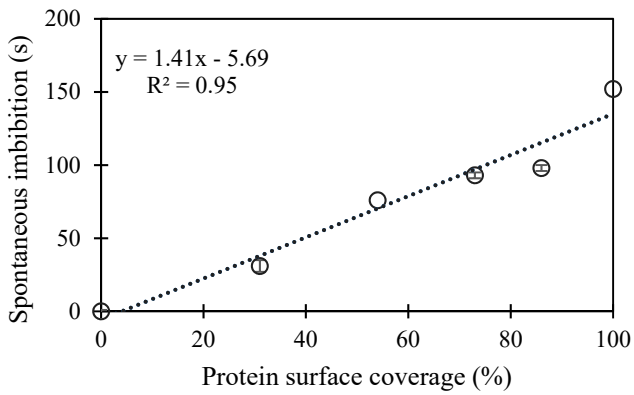

Figure 24.
The time for spontaneous imbibition (s) as a function of protein surface coverage (%) of serum protein/lactose (% w/w) powders (**Paper I, II**).

Figure 23b shows the spontaneous imbibition of the powders as a function of the protein concentration in the powders. The spontaneous imbibition of the powders was estimated by measuring the time for a water droplet to penetrate a loosely packed powder bed. As seen in Figure 23b, the spontaneous imbibition time increased with the protein concentration. More interestingly, as shown in Figure 24, the spontaneous imbibition showed a positive linear correlation with the protein surface coverage which implies that the surface composition plays an important role for the spontaneous imbibition Similar findings were observed by Nijdam and Langrish (2006) and Gaiani et al. (2010) who found that the wetting rate of SMP and WPI powders, respectively, decreased with increasing amount of proteins present at the particle surface. In addition, it was observed in this thesis that the spontaneous imbibition of serum protein/lactose (% w/w) powders 40/60 prepared at a pH of 8.6 was longer (140 s) compared to powders prepared at pH 7.3 (67 s) (**Paper V**). As the pH increases, the proteins become more soluble due to repulsive forces between the charged groups of the proteins (Pelegrine and Gasparetto, 2005). As discussed earlier, proteins that dissolve faster might result in gel formation within the inter-particle space and, thus, result in a slower spontaneous imbibition time.

In addition, by using the flow-cell based method, it was possible to distinguish an interface between the dry and wetted powder (1-60 % w/w of protein) (**Paper II**). As the protein concentration increased to 90%, the powder seemed to become totally wetted by the water. Earlier studies have shown that a powder with good wettability more easily could form a gel resulting in slower imbibition rates and slower water penetration of the powder bed (Hellborg et al., 2012). Thus, the observed interface between the powder bed and the waterfront of the powders with less protein (≤60 % w/w) might actually be caused by a good wettability of the powder. In addition, Börjesson (2015) observed that a large amount of air was entrapped in a powder bed upon imbibition. These structures with entrapped air might cause a variation in the imbibition rates as well.

Impact of protein denaturation and lactosylation in SPC/lactose powders

Unfolded/denatured proteins are expected to have poorer wettability than native proteins due to exposed hydrophobic residues as the protein loses its' tertiary structure. Thus, it was of interest to investigate how the rehydration properties was affected by protein denaturation in spray-dried serum protein/lactose (% w/w) powders. The forced imbibition was estimated by measuring the change in absorbance as the powder was added to water under gentle stirring (**Paper III, IV**). The fraction of insoluble proteins was estimated using the Bradford method (**Paper IV**). Figure 25 shows the forced imbibition rate of serum protein/lactose (% w/w) 1/99 and 60/40 powders as a function of the insoluble protein fraction. The insoluble protein fraction was varied in the powders by heating the feed between 70 to 80°C for 15 with or without additional lactose (a more detailed description can be found in **Paper III**). As seen, there is a slight reduction in the imbibition rate as the insoluble fraction of proteins increases in the powders. Even though the native fraction of proteins reduced with almost 50%, the forced imbibition rate decreases only 20-30% in both powder systems. It was expected to see larger differences in the imbibition rate of protein/lactose (% w/w) 1/99 powders compared to 60/40 as ~15% of the bulk protein would be present at the surface of these powder particles (Landström et al., 2000). Further, no differences in the insoluble protein fraction nor the forced imbibition rate could be distinguished depending on if the serum protein was heat-treated with or without additional lactose (Figure 25).

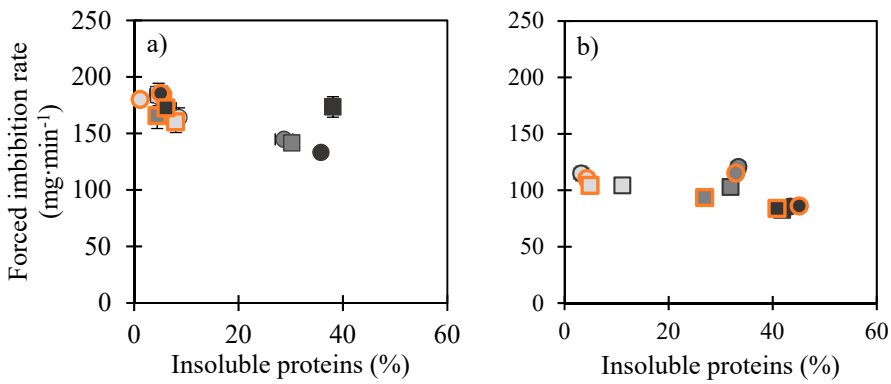

Figure 25.
Forced imbibition rate (mg·min^{-1}) of serum protein/lactose (% w/w) powders 1/99 (a) and 60/40 (b) as a function of insoluble proteins before (circles) and after storage (squares). Filling code – Heat treatment of feed for 15 s at 70°C (light grey), 75°C (medium grey), or 80°C (dark grey). Feed heat-treated with additional lactose are marked with an orange outline. The error bars illustrate the standard error of mean (n=4, n=2) (**Paper III, IV**).

In contrast to protein denaturation, lactosylation of whey proteins might improve the wettability due to increased hydrophilicity of the bound lactose molecule. To induce changes, the serum protein/lactose (% w/w) 1/99 and 60/40 powders were stored at 30°C, at a_w 0.23 for 25 days. The degree of lactosylation was estimated using liquid chromatography - mass spectrometry. After storage, the lactosylation of α-la and β-lg significantly increased between 100 and 200% (**Paper III**). Figure 26 displays the forced imbibition rate of the powders as a function of the lactosylation of β-lg. As seen, the forced imbibition rate is not affected to a large extent by the lactosylation in none of the powder systems. Further, as seen in Figure 26, the lactosylation of β-lg are more pronounced in the powders with large fraction of proteins (60% w/w) and not in the powders with a high fraction of lactose (99% w/w). When the study was designed, it was expected to observe the opposite effect where an excessive amount of lactose would result in enhanced lactosylation after storage. These findings imply that the surface composition is rather similar before and after storage of the powders as the forced imbibition rate was not affected to a large extent. Further, as a large fraction of the proteins at the surface could be expected to be native, it could be speculated that the lactosylation does not occur at the surface of the powder particle but rather in the interior of the powder particle. This has also been suggested by others (Norwood, 2016).

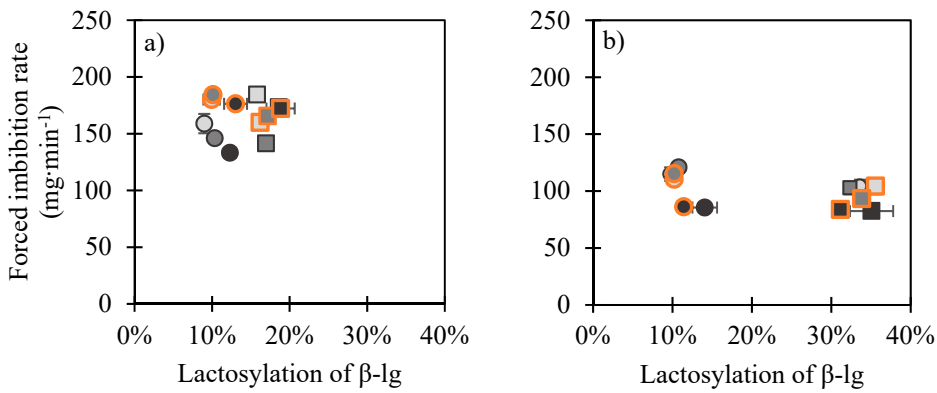

Figure 26.
Forced imbibition rate (mg·min^{-1}) of serum protein/lactose (% w/w) powders 1/99 (a) and 60/40 (b) as a function of lactosylated β-lg before (circles) and after storage (squares). Filling code – Heat treatment of feed for 15 s at 70°C (light grey), 75°C (medium grey), or 80°C (dark grey). Feed heat-treated with additional lactose are marked with an orange outline. The error bars illustrate the standard error of mean (n=4, n=2) (**Paper III, IV**).

The microstructure and the distribution of the powder constituents in the powder particles might explain these observations. It is suggested that the rate of lactosylation is higher in protein-rich domains with low amounts of lactose than in lactose-rich domains with a low amount of proteins. As seen in Figure 22, the

protein-rich domain in the vicinity of the powder particle surface tended to become thinner as the protein concentration increased. This could either indicate that the protein domains become smaller in the powder particle or that the phase segregation become less pronounced as the viscosity of the feed increases as a result of the protein concentration. However, the CRM cannot give information of the distribution of small species domains (<300 nm). Thus, it is unclear how the protein domains are distributed in the particle matrix at higher protein concentrations (≥10 % w/w). However, as shown earlier, the SEM micrographs in Figure 19 revealed that protein aggregates were present in the interior of the powder particles. Based on this, a simplified schematic overview of the segregation between protein and lactose in the powder particle is shown in Figure 27. The two domain-forming states that are separated are a glassy state of lactose with dissolved protein and a glassy state of protein with dissolved lactose. Lactose is considered to be more soluble in the glassy state of protein than protein in a glassy state of lactose. Thus, from the observations in the serum protein/lactose (% w/w) 1/99 system it can be concluded that the rate of lactosylation in the glassy state of lactose is slow (Figure 26a). On the other hand, the enhanced lactosylation in the serum protein/lactose (% w/w) 60/40 system could be caused by the formation of a glassy protein phase with dissolved lactose. Such a state could be expected to have higher mobility and, thus, a higher reactivity than a lactose glass. Indeed, higher diffusion rates of water has been observed by Maidannyk et al. (2020). However, the reactivity of the molecules highly depends on its environment, temperature, and water activity (Roos, 1995).

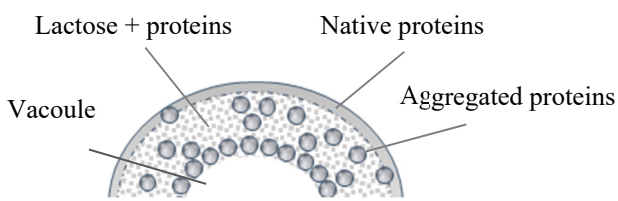

Figure 27.
A simplified schematic overview of the segregation between lactose and protein in the interior of a spray-dried powder particle. Protein enriched layer at the particle surface followed by a two-phase region with a protein-rich and a lactose-rich phase (Paper III).

Nevertheless, earlier studies have indicated that denatured proteins have more available lysine groups as a result of the loss of the tertiary structure to react with compared to native proteins (Fenaille et al., 2004, Losito et al., 2010). Hence, to achieve a better understanding of how heat treatment affects the lactosylation of the whey proteins in the powders, it would be of interest to investigate the degree of lactosylation of denatured proteins as well.

Impact of aggregation state of whey proteins

Microgels and fractal aggregates were produced by heating SPC at pH 7.3 and 8.6 with a protein and lactose concentration of 60 and 90 $g \cdot L^{-1}$, respectively, at 85°C for 15 minutes (**Paper V**). Unheated SPC was added in varying ratios to achieve systems with different degrees of native proteins. The protein/lactose ratio was kept constant (40/60 % w/w). The forced imbibition rate was estimated by measuring the change in absorbance upon reconstitution of the powder at 600 nm. The spontaneous imbibition was estimated by measuring the time for a water droplet to penetrate a loosely packed powder bed.

Table 6.
Spontaneous imbibition (s) and forced imbibition rate ($mg \cdot min^{-1}$) of microgel (prepared at pH 7.3) and fractal aggregate (prepared at pH 8.6) serum protein/lactose (% w/w) 40/60 powders with varying amount of native proteins.The standard error of mean is displayed (n=3).

Amount of native serum protein (%, dry weight)	Spontaneous imbibition (s)		Forced imbibition ($mg \cdot min^{-1}$)	
Microgels/Fractals	Microgels	Fractals	Microgels	Fractals
1.5/5.3	156 ± 12	131 ± 6	144 ± 12	126 ± 14
1.5/5.0	104 ± 8	156 ± 3	137 ± 7	133 ± 1
3.2/6.7	111 ± 5	130 ± 12	139 ± 12	134 ± 4
4.7/7.8	105 ± 2	130 ± 4	138 ± 2	146 ± 5
10.7/13.2	104 ± 6	141 ± 12	140 ± 2	161 ± 7
15.1/16.5	104 ± 3	141 ± 5	147 ± 14	192 ± 23
19.6/20.1	95 ± 9	139 ± 14	172 ± 6	243 ± 12
32.0/-	67 ± 3	146 ± 4	204 ± 13	237 ± 19

The results of the imbibition measurements and the forced imbibition rate are displayed in Table 6. The forced imbibition rate of the microgel and fractal aggregate powders exhibit similar trends. At lower native protein fractions, the forced imbibition rate is more or less constant. However, when the amount of native protein increases to ~15% the forced imbibition rate increases in both powder systems but slightly more in the fractal aggregate powders. Further, the time for the spontaneous imbibition is overall lower for the microgel powders compared to the fractal aggregate powders (Table 6). As mentioned earlier, this observation could be caused by the enhanced solubility of the proteins, which might result in a faster gelling, in the fractal aggregate powders as a result of the pH of 8.6. This phenomenon can also be seen by comparing the powders with only native proteins prepared at pH 7.3 and 8.6, respectively, where the spontaneous imbibition time is more than the double for the powder with the higher pH (Table 6). Interestingly, the time for spontaneous imbibition decreases almost 30% between the two microgel powders with the lowest amount of native proteins (from 156 to 104 s). The difference between these systems is that 1% (w/w) of native serum protein was added to the system with a faster imbibition time. As the surface composition affects the wettability it could be speculated that the composition differs between these

powders. The powder with a faster spontaneous imbibition might be less hydrophobic compared to the powder with the slower imbibition time. As shown earlier in Figure 17, denatured and aggregated proteins seem to contribute to the protein surface coverage of the serum protein/lactose (% w/w) 40/60 powders containing a small fraction of native protein (1.5-5%, dry weight). Figure 28a shows the spontaneous imbibition time as a function of the native protein surface coverage. As shown earlier, there is a linear relationship between the spontaneous imbibition and the protein surface coverage from **Paper I**. It is interesting to notice that the native serum protein/lactose 40/60 (% w/w) powder from **Paper V** had a slower spontaneous imbibition time compared to the same serum protein/lactose ratio from **Paper I**. Thus, if the particle surface of the microgel powders only consisted of native proteins, the data points should be situated more closely to the native serum protein/lactose powder below the dashed line. Figure 28b shows the spontaneous imbibition time as a function of the estimated surface coverage of denatured proteins. As seen, the spontaneous imbibition time of the microgel powders (medium-grey filling in Figure 28) increases the more denatured and aggregated proteins present at the surface. This indicates that aggregated proteins at the particle surface reduce the wettability of the powders.

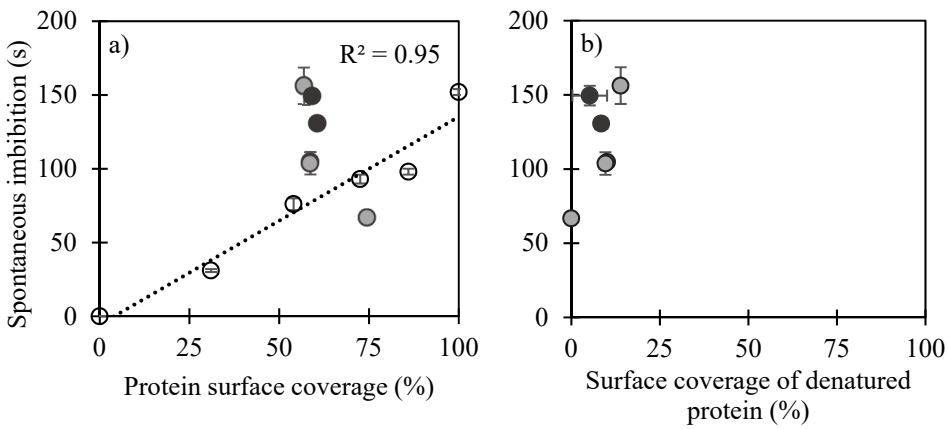

Figure 28.
Spontaneous imbibition (s) as a function of protein surface coverage (%) (a) and protein surface coverage of denatured proteins (b). Filling code – Data from Paper I (no filling), microgels from **Paper V** (medium grey), and fractal aggregates from **Paper V** (dark grey). The standard effor of mean is displayed. Protein surface coverage (n=2) and spontaneous imbibition (n=6) for data from **Paper I** and n=3 for data from **Paper V**.

Conclusions

In this thesis, spray-dried SPC/lactose powders have been investigated in varying ratios. The degree of denaturation and lactosylation have been altered and the rehydration properties have been examined. This thesis has shown:

- Possibilities to predict the particles morphology by estimating the surface rheology properties at the air/water interface of the feed droplet (**Paper I, Paper V**)
- As the protein concentration increases in the powders, it is suggested that the proteins become more densely packed at the particle surface but not necessarily result in a thicker protein layer (**Paper II)**.
- The wettability of the powders showed a linear correlation with the protein surface coverage. Denatured proteins at the surface reduced the wettability of the powders (**Paper II, IV, V**).
- An increase of lactosylated β-lg from 10% to 35% in lactose-rich and protein-rich powders, respectively, had no impact on the rehydration properties (**Paper III, IV**).
- Protein denaturation and aggregation state of the proteins had a rather limited effect on the rehydration properties of the powders (**Paper IV, V**). It is suggested that the native proteins dominate the particle surface and that the aggregated proteins are mostly found in the interior of the powder particles.

Changes in composition and solution properties as well as physical state of milk serum proteins were shown not to have a major impact on the rehydration properties. The powders were shown to be quite robust against changes in the protein structure in relation to particle morphology and rehydration properties. As all systems investigated contained a fraction of native proteins, it is suggested that the particle surface is dominated by these proteins causing the small differences. Consequently, native serum proteins and lactose have shown to be potential to protect and encapsulate less surface-active components. This thesis has contributed to further understanding of how the microstructure and rehydration properties of spray-dried whey powders are affected by the feed properties. This knowledge can be used to formulate systems with improved rehydration characteristics.

Future perspectives

This thesis has provided a better understanding on how the surface composition, particle morphology and rehydration properties of spray-dried serum protein/lactose (% w/w) powders are affected by the protein content, degree of denatured proteins as well as the degree of lactosylation of α-la and β-lg. The results indicate that serum protein/lactose (% w/w) systems are quite robust against severe treatments as the rehydration properties and particle morphology were overall only slightly affected by protein denaturation and aggregation. It seems that native serum protein and lactose protect protein aggregates in the powder matrix as they adsorb to the surface of the droplet during the drying. Thus, it would be interesting to evaluate whether this kind of system could be used to encapsulate functional ingredients used in the food industry. Further, as laboratory spray-dried particles are rather small, it would be of interest to evaluate particles spray-dried in pilot scale in order to see if similar observations can be made on larger particles as in this thesis.

For future research, it is suggested to investigate how the structure of the protein aggregates is affected upon storage of spray-dried powders as it is essential that they keep their structure in order to fulfil their functional properties. However, to avoid lactosylation and reduction of lysine, it would be of interest to formulate powders with a non-reducing sugar instead of lactose to evaluate the functional properties and how the powder is affected upon storage.

As seen in the thesis, the results suggested that a fraction of denatured proteins were present at the particle surface and contributed to the protein surface coverage in serum protein/lactose with as large fraction of denatured and aggregated proteins. As this seemed to result in poorer wettability of the powders it would be interesting to further investigate the distribution of denatured/native proteins at the surface. To further understand the distribution of native and denatured proteins at the surface of the powder particle, a protein labelling technique could be used. Such a technique was developed by Landström et al. (1999), which is based on fluorescence quenching in gaseous phase of pyrene labelled proteins. Thus, by labelling the native proteins but not the denatured proteins, more information regarding the distribution of native versus denatured proteins at the powder particle surface could contribute to a further understanding of the rehydration phenomenon.

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Livet är som mjölkpulver, det löser sig förr eller senare.

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