TALLINN UNIVERSITY OF TECHNOLOGY

Faculty of Chemical and Materials Technology

Department of Food Processing

SURFACE RHEOLOGY OF MILK SERUM PROTEIN-LACTOSE SYSTEM

- IMPACT ON PARTICLE MORPHOLOGY AFTER SPRAY DRYING
Master's thesis

KADRI KRAFT

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KADRI KRAFT

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Autor: Kadri Kraft

....
(allkiri ja kuupäev)

Üliõpilase kood: 144278KATM

Töö vastab kehtivatele nõuetele:

Supervisor: Björn Bergenståhl
Lund University, professor

....
(signature and date)

Kaasjuhendaja: Toomas Paalme
Tallinna Tehnikaülikool, Toidutehnoloogia õppetooli juhataja

....
(allkiri ja kuupäev)

Co-supervisor: Ida-Marie Andersson
Lund University, PhD student

....
(signature and date)

Co-supervisor: Maria Glantz
Lund University, Associate Professor

....
(signature and date)

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ANNOTATSIOON

Piimast ja selle komponentidest pulbrite tootmine on väga levinud. Selliste pulbrit tootmine aitab vähendada transpordikulusid, väärindada tootmise kõrvalprodukte või võimaldab piima komponente kasutada väärtusliku toidulisandina. Teisalt kaasnevad pulbrite tootmisega ja tarbimisega teatud probleemid. Sõltuvalt koostiskomponentidest võib näiteks pulbit olla töömahukas lahustada ja see võib moodustada klompe. Pulbrite lahustumisomadused sõltuvad suurel määral nende osakeste pinna koostiskomponentidest.

Antud töös uuriti piima seerumi valkude lahuseid ja piima seerumi valkude-laktoosi süsteemi pinna reoloogat. Lisaks hinnati piima seerum valkude-laktoosi süsteemi reoloogiliste omaduste sõltuvust pihustuskuivatatud osakeste morfoloogiast ning elektrolüütide mõju süsteemile.

Antud magistritöös leiti, et nii piima seerum valkude lahuste kui ka piima seerum valkude-laktoosi süsteemi pinna reoloogia sõltub valkude kontsentratioonist proovis. Mida suurem oli valkude kontsentratsioon proovis, seda madalam oli proovi pindpinevus ja suurem pinnasurve. Tulemused, mis puudutasid elastusmoodulit polnud nii selged. Kui piima seerumi valkude lahustes elastusmoodul oli suurem kõrgema valgusisalduse korral, siis piima seerumi valkude-laktoosi süsteemis olid seosed ebaselged.

Edasised tulemused näitasid seost tilga pindpinevusel 0,3 sekundit pärast moodustumist ja pihustuskuivatatud pulbriosakese morfoloogial. Pihustuskuivatatud pulbriosakestel, mille lahuse pindpinevus (78,95 mN/m) oli lähedane 17,5%-lise laktoosilahuse pindpinevusele, oli pind peamiselt sile. Nende osakeste morfoloogia oli väga sarnane osakeste omale, mis sisaldasid ainult laktoosi. Järgneva proovi, mille pindpinevus oli eelnevast madalam (68,12 mN/m), osakeste pind oli voldiline ja nendel olid väikesed mõlgid. Proovidel, millel oli valkude-laktoosi suhe 40/60 ja 60/40 oli kõige madalam pindpinevus (vastavalt 57,95 mN/m and 57,42 mN/m), olid pihustuskuivatatud osakesed kaetud suurte voltide ja mõlkidega. Pindpinevus oli antud süsteemis põhjustatud valkudest, mis olid seal kõige pindaktiivsemad koostisosad. Seega võib öelda, et valkude kontsentratsiooni ja osakeste morfoloofia vahel on seos. Antud süsteemis uuritud elektrolüütide mõju puhul selgus, et lisatud kogusel ei olnud proovide reoloogilisele omadustele märkimisväärset mõju ($F<F_{crit}$; $2,003<5,987$).

Antud magistritöö koosneb kirjanduse ülevaatest, mis annab ülevaate piima, piima seerumi, piima seerumi valkude, pihustuskuivatamise, valkude adsorptsiooni, rippuva tilga meetodi

(*Pendant drop method*) ja ostsilleeruva rippuva tilga meetodi (*Oscillating Pendant drop method*) kohta ning selles on kirjeldatud, kuidas kujuneb valgu-laktoosi süsteemi osakese pind pihustuskuivatamise jooksul. Eksperimentaalne osa käsitleb materjale ja meetodeid, katsete kirjeldust, tulemusi ja nende analüüsi, arutlust, järeldusi ning kokkuvõtet.

Töö koosneb 58 leheküljest, 14 joonisest, 11 tabelist ja 3 lisast.

ABSTRACT

Nowadays dairy powders are common products and their market share is increasing. Dairy powders are produced for various reasons, such as to reduce the transportation cost, handle seasonal variation in milk or to valorise the by-products of the production. However, powder production and consumption can entail several problems, such as inadequate solubility or the formation of lumps during wetting. The wetting properties of the powders are to a large extent dependent on the degree of agglomeration, morphology and surface composition of the powder particles. In addition, there is a hypothesis that the surface morphology is influenced by the surface rheology.

In this thesis, the surface rheology of diluted milk serum protein and milk serum protein-lactose system, has been evaluated. Furthermore, the effect of electrolytes in the system, the connection between the surface rheology of the milk serum protein-lactose system and the morphology of spray dried particles, was evaluated.

It was found that that the surface rheology of both diluted milk serum protein and milk serum protein-lactose system is dependent on the protein concentration. The higher the protein concentration is, the lower the surface tension and higher surface pressure is. The results regarding the modulus of elasticity were inconclusive. In the milk serum proteins system, the modulus of elasticity was higher for the samples with a higher protein concentration. However, in the milk serum protein-lactose system, the modulus of elasticity was higher for the samples with the lowest protein concentrations, and showed an unexpected pattern.

The results obtained for the milk serum protein-lactose system showed a correlation between the surface tension at 0.3 seconds and the spray dried particle morphology. Particles formed from a liquid with a surface tension (78.9 mN/m) near to surface tension of 17.5% lactose solution had almost a smooth surface. If the surface tension decreased (68.1 mN/m), the particles got more dented and ridges appeared on the surface of the particle. Samples with milk serum protein/lactose ratio of 40/60 and 60/40 had the lowest surface tension (respectively 57.9 mN/m and 57.4 mN/m), and the spray dried powder particles were covered with large dents and ridges. Since the decrease in surface tension is caused by proteins, which are the most surface active compounds in the system, the connection between protein concentration and spray dried particle morphology was established. In addition, it was found that electrolytes in used amount did not had significant effect on the milk serum protein-lactose system (F<Fcrit; 2,003<5,987).

The thesis has 2 main parts, namely a Literature review and an Experimental part. The Literature review gives an overview of milk, milk serum, milk serum proteins, spray drying process, protein adsorption, Pendant drop and oscillating Pendant drop method, and how the surface rheology is affecting the morphology of the spray dried powder particles. The Experimental part covers material and methods, description of experiments, results, discussion, conclusion and a summary.

The thesis consists of 58 pages, 14 figures, 11 tables, and 3 appendices.

Acknowledgements

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Kadri Kraft

Lund, May 2016

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CONTENTS

ABBREVIATIONS

α-LA - α-lactalbumin

β-LG – β –lactoglobulin

A – interfacial area

BSA – Bovine Serum Albumin

dA - the change of an area (occurs periodically)

Ig - Immunoglobulins

GMP - Glycomacropeptides

MiSP – Milk Serum Protein

SPC – Serum Protein Concentrate

SEM - Scanning Electron Microscope

R_1 and R_2 - the principle radii of curvature of the interface

ΔP - the pressure difference between the gas and the liquid phase

γ_0 – surface tension of the pure solvent (mN/m)

γ - surface tension (mN/m)

γ(t) - surface tension of the solution at time t (mN/m)

π(t) – surface pressure of the pure solvent at time t (mN/m)

ω – frequency of oscillation (Hz)

M - mass of the drop, mg

1. INTRODUCTION

Milk and its compounds are commonly produced into powders and widely used in food industries. Manufacturing dairy powders enables producers to extend the shelf life of the product, reduce the transportation costs or valorise the by-products. Spray drying is one of the most used methods for drying and producing powder from milk-originated liquids. However, the obtained powder may be laborious to dissolve; particles may aggregate and form lumps or cause other difficulties for the producers, as well as for the consumers.

Fäldt et al. (1994) discovered that the most surface active component accumulates on the surface of the particle in protein-lactose system during spray drying. Ever since, proteins as the most surface active compounds in this system have been more in focus regarding the surface composition of protein/lactose powders. It is believed that proteins on the surface of the dried particles contribute to lower solubility of the powder.

When it comes to caseins and milk serum proteins separately, they have been under investigation in numerous studies (Suttiprasit et al.; 1992, Fäldt et al. 1994; Landström et al., 2000). Milk serum proteins as a complex have been the object of research far less. In this thesis milk serum proteins have been investigated and the surface rheology of milk serum protein solutions was on focus.

1.1. Objective

The Objective of this thesis was to evaluated rheological properties of diluted milk serum protein systems, and observe how rheological properties are affected by diluting the system with lactose solution in various ratios. Furthermore, the relationship between the rheological properties of the milk serum protein-lactose system and morphology of the spray dried particles, have been evaluated. In addition, another objective was to evaluate whether the electrolyte concentration in milk serum protein system has an effect on rheological properties of the milk serum protein solutions.

1.2. Hypothesis

- Surface rheology (surface tension, surface pressure and modulus of elasticity) can be used to describe and predict the morphology of the spray dried milk serum protein solution particles.
- Lactose affects the rheological properties of milk serum protein solution.
- Adding electrolytes to the milk serum protein-lactose system will affect the surface rheology of the system.

2. LITERATURE REVIEW

2.1 Milk

Milk is an emulsion where fat globules are dispersed in an aqueous phase. In the aqueous phase essential compounds are dissolved, such as proteins, lactose, minerals and vitamins. Water is the main component in milk, and the average water content is around 87% (w/w). On average, the solid content of milk includes 4.6% (w/w) of lactose, 4% (w/w) of fat, 3.3% (w/w) of proteins, smaller amount of mineral substances (0.7% w/w), organic acids (0.17%) and other components (0.15%) (Walstra et al., 2005). Milk has a very high nutritional value and it is determined by milk composition. In addition to high macronutrient content, milk contains significant quantities of almost all micronutrients. However, the iron and vitamin C content is low and it lacks of dietary fibre (Walstra et al., 2005). The pH of milk is approximately 6.7 (Walstra et al., 1999).

Lactose, the carbohydrate in milk is a disaccharide and consist of D-galactose and D-glucose that are linked by a β-1,4-glucosidic linkage. Lactose can exist in amorphous or crystallized form (Walstra et al., 1999). Lactose is soluble in water and at 25 °C its solubility in water is 17.8 g/100 g. (Stephen et al., 1964). Lactose is easily supersaturated by increasing the temperature of the lactose-water solution (Walstra et al., 1999).

The main lipids in milk are triglycerides but other lipids are also present, such as cholesterol phospholipids, diglycerides and free fatty acids. Fats are primarily present in milk as fat globules that are surrounded by a complex membrane, called milk fat globular membrane. (Walstra et al., 1999)

Milk proteins are divided into two major groups: caseins and milk serum proteins. Caseins are the most abundant group of proteins in milk, accounting for 79.5% of the total protein content. By definition, casein is a milk protein that precipitates around pH 4.6. Caseins are rather heat-stable proteins and do not denaturate at temperatures below 100°C (Walstra et al., 2005). The casein family consists of αS1-, αS2-, β-, and κ-caseins. Caseins can form micelles - colloidal particles that consist of water, salts and proteins. Caseins are dispersed in a liquid called milk serum. The milk serum is considered as milk without fat globules and casein micelles (Walstra and Jenness, 1984; Walstra et al., 2005).

The second major group of proteins are called serum proteins. They are dissolved in the milk serum and can form small-sized aggregates. Milk serum proteins are heat-sensitive (denaturate around 80ºC) and soluble in water. These proteins remain soluble at pH 4.6, which is not the case for caseins, and they do not coagulate in acidic conditions (Walstra et al., 2005).

2.1.1 Milk serum proteins

Although milk serum proteins are similar to whey proteins and are sometimes referred to as whey proteins, they are not exactly the same. Whey proteins are obtained after cheese production from whey. In the cheese manufacturing process where whey is expelled from the formed casein gel (curd), certain fractions of κ-casein is detached and released to the whey. These biologically active components are called glycomacropeptides (GMP). The absence of GMP-s in milk serum proteins mainly distinguishes them from whey proteins (Walstra & Jenness, 1984; Neelima et al., 2013; Evans et al., 2010). In addition, milk serum proteins have not been exposed to chemical nor enzymatic reactions, as whey proteins, which may reduce the functionality of the proteins (Evans et al., 2010).

Serum proteins are primarily globular proteins, except proteose-peptone. It means that their peptide chains are compactly folded. This gives them a rather high hydrophobicity. When milk is heated (around 80 ºC), they become insoluble. However, instead of flocculating, they precipitate onto the casein micelles and stay dispersed in milk. Milk serum proteins have the ability to bind a small amount of water and counter ions. The main milk serum proteins are; β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA) and immunoglobulin (Ig). (Walstra et al., 1999)

β-LG is the most abundant protein in milk serum, accounting for 56% of total protein content. The native structure of β-LG composes of 162 amino acid residues and its molecular weight is approximately 18.3 kDa (Figure 1) (Gunasekaran & Solar, 2012). It has one very reactive free sulfhydryl group, two disulphide linkages. β-LG is present in milk as a dimer where monomers are tight to each other primarily by hydrophobic interactions. The hydrophobicity of β-LG is 5.1 kJ/, and is thereby a very hydrophobic protein. (Suttiprasit et al., 1992). β-LG is not soluble in pure water and its solubility properties depend on pH and ionic strength. Apart from nutritive function, any other special function of β-LG in milk has not yet been

found (Jenness, 1970, Kontopidis, Holt, & Sawyer, 2004). β-LG does not bind any calcium (Ca^{2+}) (Walstra et al. 1999, Walstra & Jenness, 1984).

The second major protein in milk serum is α-LA. α-LA consist of 123 amino acids which forms a single polypeptide chain. α-LA has 4 disulphide linkages that can interact with β-LG-s free thiol groups. The molecular weight of α-LA is 14.1 kDa. This protein has compactly folded structure and it associates only at low ionic strength, and is only marginally dependent on the pH and salt concentration. The biological function of α-LA is to be a coenzyme in the lactose synthesis. α-LA has the ability to bind strongly to Calcium, one mole calcium ions (Ca^{2+}) per mole α-LA (Walstra & Jenness, 1984; Walstra et al. 1999).
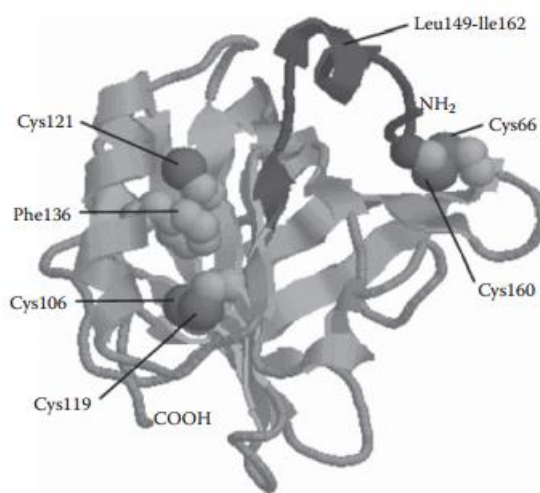
Figure 1. 3D structure of Beta-lactoblobulin (Creamer et al., 2004)

BSA is a large molecule that consists of 582 amino acid residues which are connected by a single polypeptide chain. It has 17 disulphide linkages. Its molecular weight is approximately 66, 3 kDa. It does not bind any calcium (Ca^{2+}) and is less hydrophobic than α-LA than β-LG. (Walstra & Jenness, 1984; Walstra et al. 1999).

Ig are antibodies that are naturally occurring in blood. They are synthesized by different secretory cells as a response to specific antigens. In milk, several Ig classes are present, like gammaglobulins (IgG), macroglobulins (IgA and IgM). Ig-s have a heterogeneous composition and they are rather big molecules. IgG molecule consists of two heavy (H) and two light (L) chains and its molecular weight is approximately 150 000 Da. It has two reactive sites that are identical. The antigen binds to the specific site through a several interactions,

such as hydrogen bonds, electrostatic attraction and hydrophobic bounds. IgG like many other immunoglobulins engage many antigens. (Walstra et al. 1999)

2.1.2 Production of milk serum proteins

Milk serum proteins are separated directly from milk by removing milk fat and micellar casein (Figure 2). Milk lipids are removed from milk by centrifugation. The obtained skim milk is suitable for separation of casein. The separation of casein is based on the size difference of milk serum proteins and casein micelles. Casein micelles are larger than serum proteins, respectively 0.2 and 0.01 μm. Micelles are separated by microfiltration using ceramic membrane with a pore-size diameter of 0.1 μm. Microfiltration is performed around 50°C. The retentate obtained after microfiltration (MF) is suitable for production of Serum Protein Concentrate (SPC). Serum milk protein separated by microfiltration can be called different names, for example, virgin whey proteins, milk serum proteins, and native whey proteins. Microfiltration is followed by ultrafiltration at lower temperatures (around 15°C). Ultrafiltration separates milk into 2 parts - concentrate and permeate (Walstra & Jenness, 1984). Milk serum protein concentrate can thereafter be spray dried to produce powder.
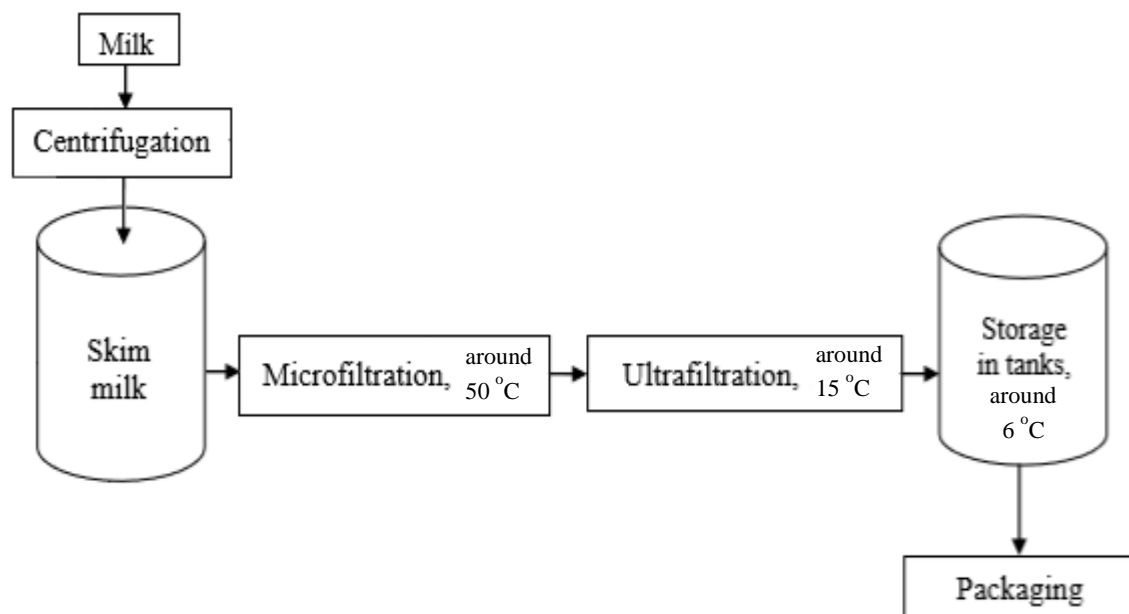

Figure 2. Production plan of Milk Serum Protein concentrate (modified after, Arla Foods, 2016).

2.2 Spray drying

Spray drying is a widely used process in milk powder production, and it is used to remove water from the feed to produce milk powders. Water is removed from the milk concentrate in a rapid process where heat exposure to the particles is minimal. (Kim et al., 2009)

Spray drying involves 3 main steps: atomization, dehydration and collection of the powder (Ameri & Maa, 2007). In figure 3, a schematic overview of the spray drying process is displayed. The feed is atomized into small droplets by a two-fluid nozzle (1) inside of a drying chamber. In the drying chamber, the small droplets get into contact with hot dry air (2). Very fine atomized droplets ensure that the contact area between the hot air and the droplet is large enough. The inlet temperature of the hot dry air is usually around 180 to 220 °C (Kim et al., 2009). When the droplet is exposed to the hot air, a saturated vapour film is formed around the droplet. The length of the dehydration of the particles is less than a second. In the third step, particles are passed on to a cyclone. Inside the cyclone (3), the particles are carried by a centrifugal force into a product collector (4). (Ameri & Maa, 2007)

1 – Two-fluid nozzle that atomozes the feed
2 – Dring chamber where droplets get into contact with hot dry air
3 – Cyclone that channels the particles to product collector
4 – Product collector
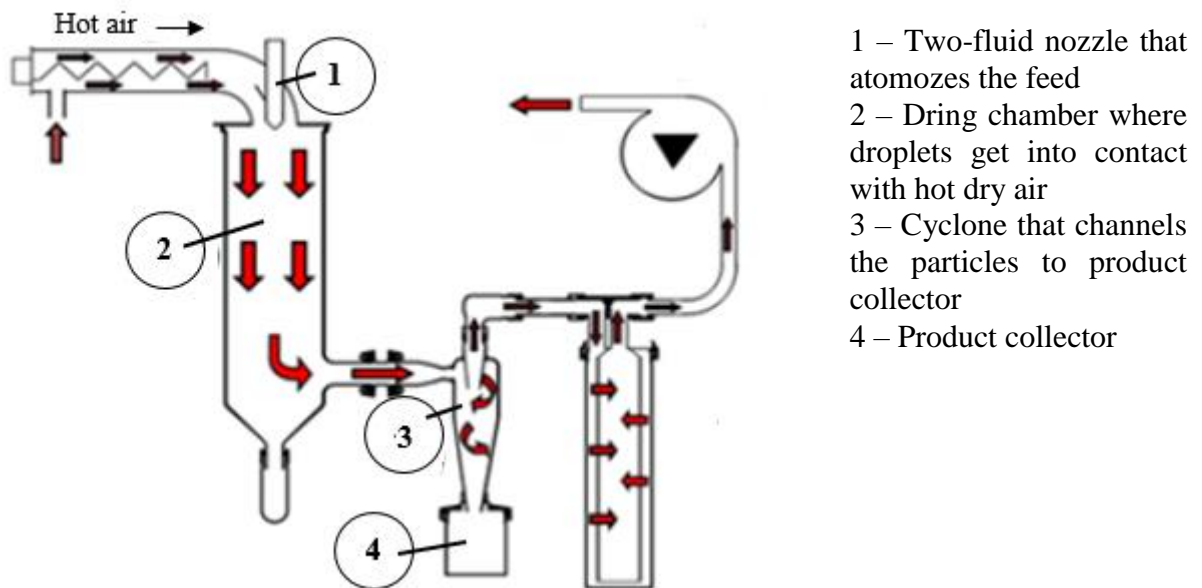
Figure 3. Scheme describing the construction of the spray drier (Modified from Büchi, 2016)

According to Charlesworth et al. (1960), the drying process (Figure 4) can be described through 2 periods. At the beginning of the first period, the droplet is a liquid and the solvent can easily move from the inside towards the surface of the droplet. The free water migrates from the centre of the droplet to the droplet´s surface and evaporates quickly. As water

evaporates, the solutes get more concentrated in the centre of the droplet which causes a concentration gradient of the solutes. Since the evaporation of the solvent at the surface takes place at a constant rate, this stage is called the constant rate period. During this stage, the radius of the droplet decreases, the droplet shrinks. The evaporation of water lowers the temperature of the droplet, and the temperature of the material will therefore not exceed the wet bulb temperature of the drying air. The droplets turn into particles when the moisture content in the upper layer becomes too low to keep the conditions saturated, dissolved substances solidify on the surface, and an adsorbed layer is formed on the surface of the particle. While the layer is thickening, the drying rate decreases and the temperature of the particles increases. This drying stage is called the falling rate period. (Charlesworth & Marshall, 1960; Kim et al., 2009)
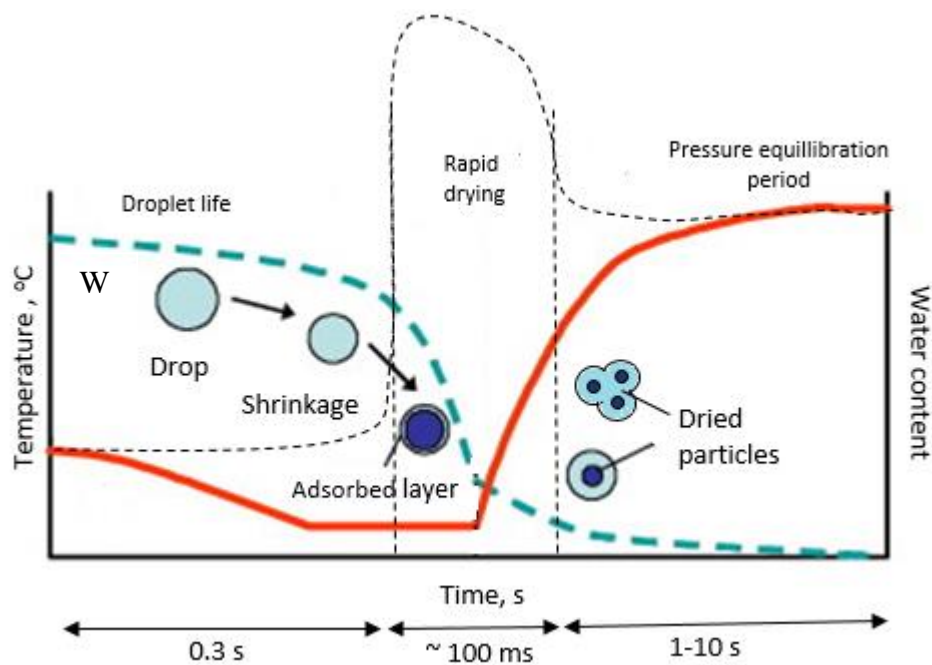
Figure 4. The drying of the droplet and temperature change as a function of time schematically (Modified after Kim et al., 2009). 0.3 seconds corresponds to lifetime of the droplet.

The main spray drying process parameters are: drying air inlet temperature, drying air outlet temperature, drying air flow rate, atomizing air flow rate and residence time (Ameri & Maa, 2007).

2.2.1 The impact of proteins on surface composition of the protein/lactose powder particles during spray drying

The surface composition of the spray dried protein/lactose powder particles is largely dependent on the surface active compounds. Research conducted by Fäldt et al. (1994) demonstrated that the surface composition of the protein-lactose spray dried powder was determined by the surface activity of the feed components. Proteins as the most surface active compounds in the protein-lactose system migrate to the surface. Proteins accumulate on the surface of the powder particles even at low concentrations. In a lactose/albumin system where protein concentration was 0.01%, the surface coverage was 3.0 As the protein concentration increased, the protein coverage on the dried particle surface increased (a protein/lactose ratio 5/95 (w/w) had a protein surface coverage of 65%). In addition, it was found that when the protein concentration is even higher in the feed, the presence of proteins on the surface of the powder was widespread (Fäldt & Bergenståhl, 1994). The Authors proposed that proteins, as the most surface active compounds in the solution, accumulate at the air-water interface.

Porowska et al (2015) investigated also the accumulation of the compounds on the surface of the powder particle during spray drying. The Authors used whey protein isolate in this work. Porowska et al. (2015) proposed that accumulation of the whey proteins at the surface may be connected to the surface activity of the feed solution. The adsorption of the surface active components to the interface can be observed through a decrease in surface tension. They also found that the surface tension of a 20% (w/w) protein solution decreased when the age of interface and/or protein concentration increased. The higher the concentration of the surface active compounds (proteins), the faster was the decline of the surface tension of the feed solution. Moreover, alterations in the surface composition can only occur when molecules are free to move inside the droplet. This means that the surface activity caused movement and changes in the surface composition of the droplet before the dried particles had formed. (Porowska et al., 2015)

During spray drying, the state of lactose changes from crystallized to amorphous form. It means that lactose is in glassy state. This is mainly due to rapid drying process. The properties of amorphous lactose are more similar to properties of crystalline sugar (Walstra et al., 1999). If amorphous lactose is in a matrix with macromolecules, such as proteins, a phase segregation is likely to occur. It means that proteins adsorb to the surface and form a separate

layer around the lactose matrix (Figure 5). This may lead to even thicker layer of proteins than only just diffusion process could have caused. (Nuzzo et al., 2015)
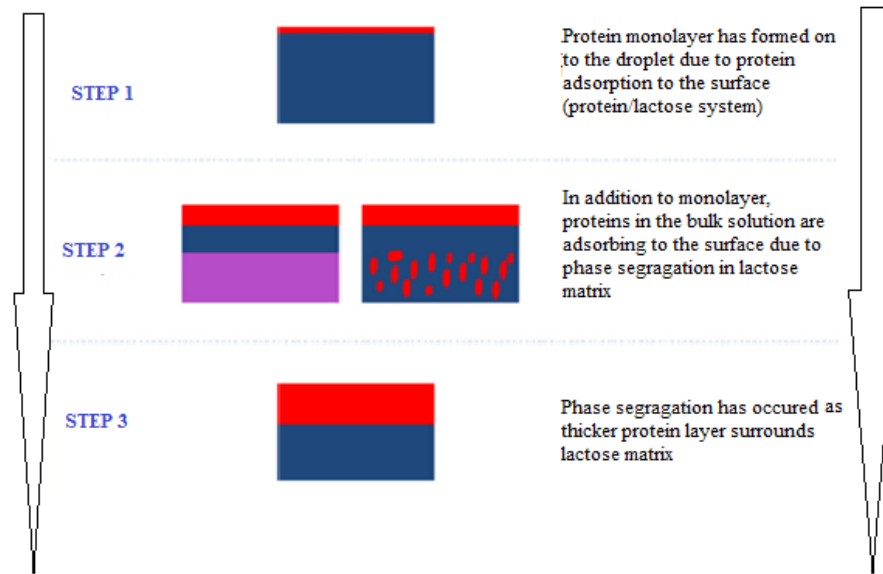
Figure 5. Scheme describing the adsorption of macromolecules to the surface in protein-lactose system resulting as 2 separated layers (Modified from Nuzzo et al., 2015).

2.3 Protein adsorption and surface activity

Proteins are one of the largest molecules in the nature, as their size ranges from 1 to 100 nm. The primary structure of proteins consists of amino acid residues that are linked together by a polypeptide chain (Tassel, 2006; Ybert & di Meglio, 1998). Secondary and tertiary structure is formed by special folding of the chain and this determines their biological function. (Haynes & Norde, 1994)

Proteins are mostly surface active biomolecules, and the surface activity of the proteins exhibits their tendency to migrate towards the surface (Xu et al, 2012). It means that in a non-surface active solution, proteins accumulate spontaneously to the droplet's interface. The accumulation of proteins at the interface is favorable due to the fact that it minimizes the surface energy. This process is necessary for the molecule to obtain energetically the best conditions. (Hlady et al., 1999)

The surface activity of proteins is determined by their composition. Proteins are built up by amino acids which can be either polar or non-polar. Dependent on the amino acids composition, the proteins have different properties. Polar amino acids are situated outside the protein globule, whereas non-polar amino acids are situated inside of the protein globule (Hlady et al., 1999). This in turn gives proteins their amphiphilic nature – one part of the molecule has hydrophilic properties while the other has hydrophobic properties. If the (hydrophobic) monomers of the proteins are attracted strongly enough to the surface, depending on the interactions between the proteins and the adsorbent, adsorption occurs. (Malmsten, 2003)

There are several forces that promote protein adsorption, such as Coulombic, van der Waals forces, hydrophobic interactions and electrostatic interaction. Electrostatic interaction takes place between the oppositely charged adsorbent and amino acid chains. It causes a noteworthy free energy change which contributes to protein adsorption process. (Haynes & Norde, 1994; Hlady et al., 1999)

Protein migration to the surface occurs through convection or diffusion. During the migration, proteins are in their native flexible or globular state. After adsorption or penetration has taken place, proteins anchor onto the surface with their hydrophobic groups or other (low-energy) fragments that are more attracted to the interface. Thereafter, the proteins rearrange at the interface, and start to penetrate the interface, unfold and spread within the surface layer to increase the amount of contact points with the adsorbent. This allows them to obtain the lowest possible surface free energy. (Fainerman & Miller, 1998; Xu et al., 2012)

Adsorption can cause surface-induced protein denaturation (Hlady et al., 1999). It means that the hydrophobic part of the protein is attracted to the interface which causes unfolding of the protein. The structure stability of native protein is dependent on complex of forces, such as van der Waals interactions, hydrophobic interactions of the hydrophobic side chains, hydrogen bonds that are between adjacent side chains and around polypeptide chains, and Coulomb´s interactions between charged residues. The surface of the adsorbent is rivalling for the same interactions that keep the structure stable. By unfolding the protein structure, the total free energy of the system is reduced. If a protein-surface contact is obtained, then the solvent molecule is released. There is a certain resistance to this but this has a rather weak effect. (Ybert & di Meglio, 1998; Hlady et al., 1999; Malmsten, M. 2003)

Because of the rather large size of proteins, the adsorption can take several hours before it reaches an equillibrium (Ybert & di Meglio, 1998). It means that protein adsorption is a time-dependent process. In addition to time, adsorption of the protein molecules is dependent on their concentration. (Rühs et al., 2013)

2.3.1 The kinetics of protein adsorption

The kinetics of protein adsorption can be described in 4 stages. This description assumes that there is an uninterrupted supply of proteins (Almeida et al., 2002; Radke et al., 2007). In the first stage, proteins adsorb onto the surface with a rapid rate which is called a mass-transfer-limited stage. The second stage of protein adsorption kinetics is the non-linear stage. In the second stage, a monolayer is formed. When a monolayer is formed, the adsorption rate decreases, which is due to smaller amounts of available free adsorption sites on the surface. Protein adsorption can get irreversible during first and second stages of the adsorption. In the third stage, the monolayer of the proteins is formed and the fourth stage is reached when multiple layers are formed. Multilayer adsorption is a rather slow process. The interactions between unfolded surface proteins and proteins in the bulk solute are rather weak because

surface proteins are minimally unfolded at the surface and available proteins at the bulk solution are mainly in their initial native conformation. In the course of time, when the proteins in the monolayer unfold further, it is thought that these interactions become larger. Protein adsorption is not fully understood and cannot be precisely predicted, mainly due to certain protein behaviour, such as the modifications in the conformation, the irreversibility of adsorption and occurring aggregation in the multilayer. (Radke et al., 2007)

2.3.2 Protein adsorption to the air/droplet interface during drying

Protein adsorption to the air/water interface during drying involves 3 steps. Firstly, molecules diffuse from the main solution to 'subsurface region'. Thereafter, the proteins migrate from the subsurface region and adsorb to the air-water interface. Finally, rearrangement of adsorbed molecules within the surface layer takes place. It is thought it happens due to the rapid spray drying process (MacRitchie & Alexander, 1963; Landström et al., 2000). The approximate time for molecule diffusion to the air-water interface during spray drying, considering the lifetime of the droplet, is 0.3 seconds (Figure 4) (Fäldt et al. 1995).

2.3.3 Morphology of protein/lactose powder particles – connection with protein adsorption and surface rheology

During spray drying, particles can agglomerate, collapse, expand, their shape can be irregular, and drying can cause holes in the shell (Nuzzo et al., 2014). The morphology of the dried powder particles can differentiate remarkably. Nuzzo et al. (2014) described morphological characteristics presented on particles as ridges, dents and wrinkles. 'Ridges' was used to describe 'large protruding folds on the surface', and 'wrinkles' to describe 'small and less-protruding folds' (Nuzzo et al., 2014).

The morphology of the powder particles are affected by the protein concentration. Fäldt et al., (1999) found that the structure of the powder particles with higher protein content was less spherical and more dented. The structure of powder particles with proteins on the surface is very different from powder obtained from pure lactose where the surface of the powder particles was entirely smooth, without any dents, ridges or wrinkles (Fäldt & Bergenståhl, 1994). On a subsequent research Fäldt & Bergenståhl (1996) indicated that the amount of dents on the surface of the particle is higher when the protein coverage of the surface increases.

The surface rheology of the droplet may determine the morphology of the dried particle. Several authors have investigated the connections between surface rheology and particle morphology. Elversson et al. (2006) assumed, based on their results, that the elastic properties of the solution have an impact on the morphology of the dried particles. Rühs et al. 2013 investigated the dilatational modulus of β-LG by oscillating Pendant drop method. The measurements of the solutions were performed by using air and oil as an interface. The results showed that the rheological properties are affecting the protein adsorption. Due to the fact that the results were inconclusive the Authors stated that improved models and measuring techniques are needed to clearly understand the adsorption and relaxation processes that are taking place at the interface during the dynamic measurements. (Rühs et al., 2013)

However, Fäldt et al., (1999) and Nuzzo et al. (2014) found some correlations between surface rheology and morphology of spray dried powders. Nuzzo (2015) compared the dilatational modulus of the droplet and scanned electron microscope images of the spray dried particles in her work. The authors proposed that the more elastic the droplet is, the more elastic is the film that forms around the droplet during drying. This in turn leads to a situation where the drop will not break during drying, and is less susceptible to contraction when the drop starts to shrink. In the other hand, the droplet that is much less elastic has a surface that can contract which leads to a smooth particle where the diameter of the droplet has decreased due to water evaporation. The Authors demonstrated that the molecules that create a soft surface, in this work polaxamer, have a low dilatational modulus. The powders containing poloxamer had a smooth surface after drying. Biomolecules that are less flexible and less prone to contractions, like BSA, have a high dilatational modulus and as a result, the spray dried particles have dents and ridges on the surface. (Nuzzo et al. 2014)

2.4 Pendant drop method and measurement

The pendant drop method is one of the most widely used drop shape measurement techniques. It is used to measure interfacial and surface tension (Saad et al., 2011). Word 'pendant' in the name of the method indicates that the surface tension measurement is conducted with a pear-shaped drop that is hanging down. The drop is formed commonly by an automatic syringe on to the tip of the needle. The surrounding environment of the formed droplet is either liquid or a gaseous phase. The shape of the drop is resulted from two opposite forces - gravity and surface tension of the drop. Gravity, in a relation to density, gives the drop an elongated shape, while the surface tension contributes to a more spherical shape of the drop (Teclis; Krüss, 2016). If a sample contains surface active substances, the surface tension of the formed droplet decreases with time (Badran & Marschall, 1986).

For surface rheology measurements with the pendant drop method, special tensiometers are used. A simplified schematic picture of the tensiometer is shown in Figure 6. This equipment include the following parts; measurement cell, camera, light source, optical stand, electric cabinet and computer for data saving and data analysis. The measurement cell consists of a syringe motor, a syringe and a cuvette. (Teclis, 2016, Berry et al., 2015)
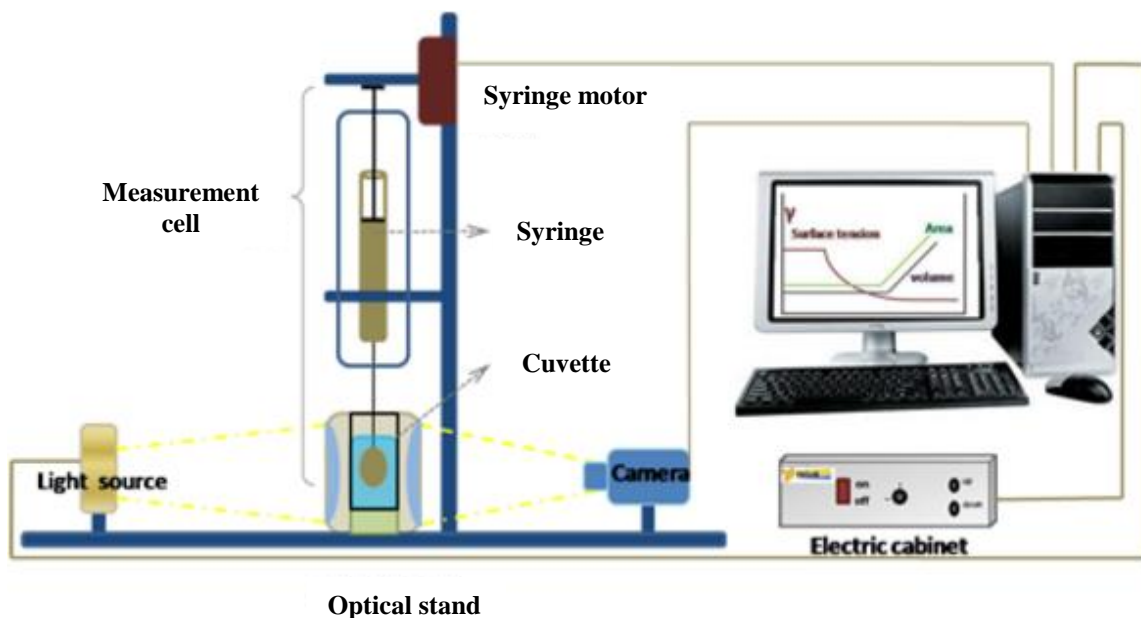

Figure 6. Basic setup of a tensiometer (Teclis, 2016).

This measurement is based on drop shape image analysis (Berry et al., 2015). The geometrical shape of the drop (the liquid-vapour interface) is used as the object for the measurements (Hoorfar et al., 2006). It is well known that the geometry of the interface can be determined by the following Young-Laplace equation (Equation 1):

$$\sigma_{LV} \left(\frac{1}{R_1} + \frac{1}{R_2} \right) = \Delta P \qquad \text{(Equation 1)}$$

where

R_1 and R_2 - the principle radii of curvature of the interface

ΔP is the pressure difference between the gas and the liquid phase which is caused by the weight of the drop (Laplace pressure across the interface)

In equation (1), the force balance impact on the liquid-vapour interface is displayed, and this can either be solved analytically or numerically. The calculation must use the actual dimensions of the drop. The actual dimensions are obtained by a real-time video image, using greyscale analysis. (Krüss, 2016)

The obtained dynamic surface tension values can be recalculated to surface pressure. Surface pressure shows the difference between the surface tension of the pure solvent and the surface tension of the analysed sample at certain time. This is described by the following formula (Suttiprasit, 1992):

$$\pi(t) = \gamma_0 - \gamma(t), \qquad \text{(Equation 2)}$$

where

γ_0 – surface tension of the pure solvent, mN/m

$\pi(t)$ – surface pressure of the pure solvent at time (t), mN/m

$\gamma(t)$ - surface tension of the solution at at time (t), mN/m

2.4.1 Advantages and disadvantages of Pendant drop method

The pendant drop method is one of the most frequently used method for surface tension measurements. However, it has few important disadvantages. The sensitivity and the performance are largely affected by the shape of the droplet. The more spherical the shape of the droplet is, the less sensitive is the measurement. The precision of the surface tension measurements is also very reliant on droplet shape and the way the experiment is conducted. In those cases where surface tension has much greater impact than gravity, the drop tends to

be more round-shaped, spherical. This leads to a situation where remarkable change in the surface tension does not cause a noticeable change in the shape of the drop. (Saad et al., 2011)

2.5 Oscillating Pendant drop measurement

Oscillating pendant drop measurement is similar to the initial pendant drop method for surface tension measurements. Even the same equipment can be used. In this case, however, the drop oscillates while hanging downwards. The measurement is based on formula developed by Rayleigh (1878) which describes the relationship between the surface tension and the frequency of oscillation for a spherical droplet (Badran & Marschall, 1986).

$$\omega_l^2 = l(l-1)(l+2) \cdot \frac{4\pi}{3}\frac{\gamma}{M} \qquad \text{(Equation 3)}$$

where

ω – frequency of oscillation, Hz

$l + 2$ – integer

γ - surface tension, nM/m

M - mass of the drop, mg

If $l = 2$, it is the lowest frequency and is called the Rayleigh frequency

Oscillating Pendant drop measurements can be used to obtain the surface dilatational elasticity modulus of the sample (Aske et al., 2002). In the oscillating Pendant drop method, the equilibrium of the droplet is disturbed by automatically controlled compression and expansion of the liquid-air interface. The measurement takes place at a certain amplitude and frequency. The measurement determines the surface tension and the surface area by analysing the shape of the drop (Shrestha et al., 2008). The area change during the compression and expansion determines the surface dilatational modulus (Lucassen et al, 1972). This is described by Gibbs equation, see Equation 4:

$$E = \frac{d\gamma}{dA/A} \qquad \text{(Equation 4)}$$

where

γ – surface tension, mN/m

A – interfacial area

dA - the change of an area (occurs periodically)

In the measurement, the elastic modulus is calculated from the complex modulus E that is made up of a real part E', and imaginary part E''. E' is the storage modulus (modulus of elasticity) and E'' is the loss modulus. The exact values of the storage and loss modulus are obtained through Fourier transformation. (Loglio et al., 1986; Rühs et al., 2013)

To obtain reliable results, the oscillation equipment requires a high-speed and accurate recording system for the calculation of the surface oscillation of the droplet (Matsumoto et al., 2004).

3. EXPERIMENTAL PART

The experimental part of the thesis is divided into 3 main sections:

Section 1: Obtaining the surface tension and modulus of elasticity values of MiSP solutions with different protein concentrations.

Section 2: Obtaining the surface tension and modulus of elasticity values of solutions with a different lactose/MiSP ratio (with and without electrolytes).

Section 3: Comparison of the surface rheology of the MiSP/lactose systems with the morphology of the spray dried particles of the solutions.

3.1 Materials and methods

3.1.1 Materials

Milk Serum Protein (MiSP) concentrate was used to prepare the samples of the experiments. It was received from the dairy company Arla Foods Ingredients Group P/S (Nr. Vium, Denmark). The protein concentration of non-diluted Milk Serum Protein concentrate was 17.5%. The composition of milk serum proteins were: 11% of β-LG, 3% of α-LA, 3% of BSA and Ig (ARLA Foods Ingredients, Denmark). The Milk Serum Protein concentrate contains 2% of lactose. The composition can be seen in Table 1.

Author of this master´s thesis points out that subsequently 'Milk Serum Proteins' is abbreviated to MiSP to make the experimental part more comprehendable for the reader.

Table 1. Composition of Milk Serum Protein (MiSP) Concentrate (Arla Foods Ingredients, Denmark).

Composition	Unit (%)
Ash	0.77
Fat	0.09
Protein	17.5
Lactose	1.97
Potassium	0.183
Calcium	0.1
Chlorine	0.04
Sodium	0.0408
Phosphorus	0.0576
Dry matter	21.88
pH	6.48

Ultrapure MilliQ (Merck Millipore Corporation) water was used for calibration and as diluent in all experiments.

Lactose monohydrate (Merck KgaA, Darmstadt, Germany) was used to prepare the MiSP/lactose solutions in Section 2 of the experiments.

As a materials were used 3 different salts:

KOH (Merck KGaA, Darmstadt, Germany) was used to prepare 0.05 M KOH solution to adjust the pH of the samples in the Section 2 of the experiments.

$CaCl_2$ (Sigma-Aldrich, Missouri, United States) was used to prepare 0.1 M $CaCl_2$ solution in the second part of the experiments for adjusting the free Ca^{2+} content in the samples.

KCl (Sigma-Aldrich, Missouri, United States) was used in Section 2 of the experiments to prepare 1 M electrolyte solution.

Deconex 53 PLUS (Borer Chemie AG, Zuchwil, Switzerland) was used to remove the impurities inside of a cuvette and a syringe that might affect the measurement.

3.1.2 Methods

3.1.2.1 Pendant drop and oscillating Pendant drop method using TRACKER^{TM}

Pendant drop method was used in all experiments to obtain surface tension of the samples. Oscillating Pendant drop was used to obtain the modulus of elasticity in all of the samples. In both cases, automated tensiometer TRACKER^{TM} (Teclis, Lyon, France) was used. The surface tension and oscillation measurements of the droplet were conducted by using air as an interface. It means that surface tension modulus of elasticity was measured in air-liquid interface.

The preparation of surface tension and oscillating Pendant drop measurements were performed the same way. Firstly, the cuvette, syringe and needles were purified with Deconex and MilliQ water. Secondly, a calibration of the equipment with MilliQ water was carried. This involved focusing, vertical and volumetric calibration. The calibration measurement was carried out for 200 seconds to verify the stability and precision of the measurement. After calibration MilliQ water was replaced by the most diluted sample and the syringe was once again place into the equipment. Before the measurement, the time of the measurement was changed from 200 seconds to 2400 seconds and density of the water was changed to the density of the sample. The density of the sample was measured and marked for every sample separately and marked as 'drop density'. In the end it was checked that the shape of the droplet was symmetrical and suitable for the measurement. For oscillating Pendant drop

measurements additional setup parameters were selected and it was marked that oscillation should start 10 seconds after the measurement has started. All the setup parameters used for the surface tension and oscillating Pendant drop measurements are shown in Appendix (Table 12 and 13).

After checking all the required parameters and shape of the drop, the measurement was started. During all the measurements, temperature was controlled with a circulating water bath. All of the measurements were carried out at 25 °C, and tap water was used as a cooling agent.

Duplicates of both the surface tension and the oscillating Pendant drop measurements were conducted. The duration of one measurement was 40 minutes (2400 s).

WINDROP software (Teclis, Lyon, France) was used for saving and analysing the data. The data was later resaved to excel worksheets. To obtain the elasticity modulus of the oscillation measurements, additional data analyses were performed. 2 cursors were moved (green and red), whereas the green was moved to the beginning of the first oscillation period (starting from 10th second), red was moved to the end of the same period. The 'harmonic button' was pressed, and it was checked if the harmonics corresponded to what the manufacture required. Finally button GO was pressed and the elasticity modulus could be obtained.

The surface tension was later calculated into surface pressure. Surface pressure was calculated by subtracting surface tension values from the surface tension of the reference solution as it was described in Literature review (see equation 2). Reference solution was pure water. The surface tension of pure water at 25 °C is 71.97 mN/m (Haynes (eds), 2015-2016).

3.1.2.2 Spray drying

Spray drying of all the prepared samples was carried out by I.M Andersson (*To be published*). The drying experiments to obtain the powder were performed using laboratory-scale spray drier Büchi Mini Spray Drier B-290 (Flawil, Switzerland). Parameters used during all of the spray drying experiments are shown in Table 2.

Table 2. Main spray drying parameters used in all drying experiments.

Inlet temperature	170 °C
Outlet temperature	80°C
Air flow (Co-current)	30 kg/h
The flow meter controlling the compressed air	About 45 mm (0.8 m^3/h)
Feed temperature (before spray drying)	between 40 °C and 45 °C

3.1.2.3 Scanning electron microscople (SEM)

Scanning electron microscope (SEM) model JSM-6700F (Tokyo, Japan) was used to capture the morphology of the particles. For obtaining the images, powders under exmination were mounted on aluminium stubs and sputter-coated with a mixture of palladium and gold (Pd-20%; Au-80%). Thickness of the metal layer was around 15 nm. A LEI detector was used. The SEM images were obtained under high vacuum using 10 kV accelerating voltage.

Parameters of micrographs: magnification: x2,000; 10 μm (The Author: I-M. Andersson, *to be published*)

3.1.2.4 Statistical analysis

Statistical analysis of the obtained data was performed using Data Analysis tool in Microsoft Excel (Microsoft Corporation). Statistical tests carried throught were Anova, F-test and T-test.

Section 1: The surface tension, surface pressure and modulus of elasticity of MiSP solutions with different protein concentrations.

The initial MiSP concentrate (**17.5%**) was diluted with ultrapure MilliQ water to obtain 5 samples with different protein concentration (Table 3). All the solutions were prepared by weight, and for this an analytical weight (accuracy of 10^{-4} grams) was used. pH was measured in every sample. pH measurements were conducted with pH-meter Metrohm 744 (Metrohm AG, Herisau, Switzerland). After the solutions had been prepared, the density of the samples was measured. For this, 1 ml of the sample was weighted on an analytical scale. This was repeated three times for each solution. Surface tension and oscillation measurements of MiSP solutions with different concentration were conducted. Surface tension was measured for all samples and two replicates were done for all 6 samples (0.000175%-17.5%). Oscillating Pendant drop measurement was done for samples containing a protein concentration of 0.175%-17.5%. In this case, four replicates were done for each sample.

Table 3. Milk serum protein concentration (%) in the diluted samples.

Milk serum protein sample	Protein concentration, %
Sample 1	0.000175%,
Sample 2	0.00175%,
Sample 3	0.0175%,
Sample 4	0.175%
Sample 5	1.75%

Section 2: The surface tension, surface pressure and modulus of elasticity values of solutions with a different lactose/MiSP ratio (with and without electrolytes).

Two sample sets with six different MiSP/lactose ratios were prepared. Their only difference was the amount of electrolytes. All of the solutions were prepared by weight. An analytical weight (accuracy of 10^{-4} grams) was used.

Lactose monohydrate was mixed with MilliQ water to obtain a lactose solution with the proper lactose concentration (Table 4). The obtained lactose solution was heated to 40°C to entirely dissolve the lactose.

Table 4. Milk Serum Protein concentrate and lactose solution ratios and measured amount of each.

Protein in SPC (g)	Lactose in SPC (g)	SPC (g)	Lactose solution (g)	Amount lactose total 1 L (g)	Amount lactose monohydrate in 1 L (g)	Total Solids (TS) in 1 L (g)
0.0175	0.00197	**0.1**	**99.9**	174.9	184.2	175
0.175	0.0197	**1**	**99**	174.8	184.0	175
0.875	0.0985	**5**	**95**	173.9	183.1	175
1.75	0.197	**10**	**90**	172.8	181.9	175
7	0.788	**40**	**60**	161.9	170.4	175
10.5	1.182	**60**	**40**	145.5	153.1	175

To keep the pH of the solutions in the range of 6.4-6.6, a pH adjustment of the lactose solution was necessary. It was done because the pH of the initial MiSP concentrate was 6.48. The pH of the lactose solution was adjusted using 0.05 M KOH solution. The pH measurements were conducted with a pH-meter Metrohm 744 (Metrohm AG, Herisau, Switzerland). The free calcium (Ca^{2+}) content was kept at a constant level in every sample by adding 0.1 M $CaCl_2$ solution. The amount of added $CaCl_2$ is presented in Tables 5 and 6, respectively with and without added electrolytes. Free Ca^{2+} concentration in the MiSP concentrate was 4 mMol/L.

After the pH adjustment and $CaCl_2$ addition, the lactose solution was mixed with MiSP concentrate according to Table 5. To one sample set (6 samples with different MiSP/lactose ratio), electrolytes were added. 1M KCl solution was added so the conductivity of the MiSP/lactose solutions would be the same as in the initial MiSP concentrate, which was around 5.50 mS. For measuring the conductivity, a portable conductivity meter ORION model 150 (Thermo Fisher Scientific Inc., Waltham, MA USA) was used. The density of the all samples was measured before the rheology measurements. For this, 1 ml of the sample was weighted on an analytical scale. This was repeated three times for each solution. After adding all compounds, the pH was measured in every sample again to verify that the pH of the sample had not gone out of the range.

Table 5. The amount of added compounds to different MiSP/lactose solutions and density and pH of obtained solution (without added electrolytes).

MiSP/lactose ratio	0.01/99.99	0.1/99.9	1/99	5/95	10/90	40/60	60/40
m(KOH), g	0.918	1.07	1.02	1.07	1.07	1.02	0.918
Conductivity, mS	5.55	1.21	1.22	1.96	2.27	2.97	3.68
V(CaCl₂), ml	0.400	0.400	0.396	0.380	0.360	0.240	0.160
m(MilliQ), g	3.30	2.19	2.36	2.32	2.03	1.47	1.28
Density ρ (g/ml)	1.17	1.15	1.15	1.15	1.15	1.12	1.11

pH	6.45	6.43	6.45	6.52	6.42	6.52	6.58

Table 6. The amount of added compounds to different MiSP/lactose solutions and density and pH of obtained solution (with added electrolytes).

MiSP/lactose ratio	0.01/99.99	0.1/99.9	1/99	5/95	10/90	40/60	60/40
m(KOH), g	0.918	0.978	0.969	0.612	0.816	0.561	0.357
V(CaCl2), ml	0.400	0.400	0.396	0.380	0.360	0.240	0.160
m(KCl), g	3.31	2.32	2.36	2.32	2.03	1.47	1.28
Conductivity, mS	5.55	5.48	5.50	5.45	5.51	5.48	5.49
Density ρ, g/ml	1.18	1.12	1.14	1.09	1.13	1.12	1.14
pH	6.45	6.52	6.49	6.40	6.40	6.67	6.53

4. Results

Section 1: The surface tension, surface pressure and modulus of elasticity of MiSP solutions with different protein concentrations.
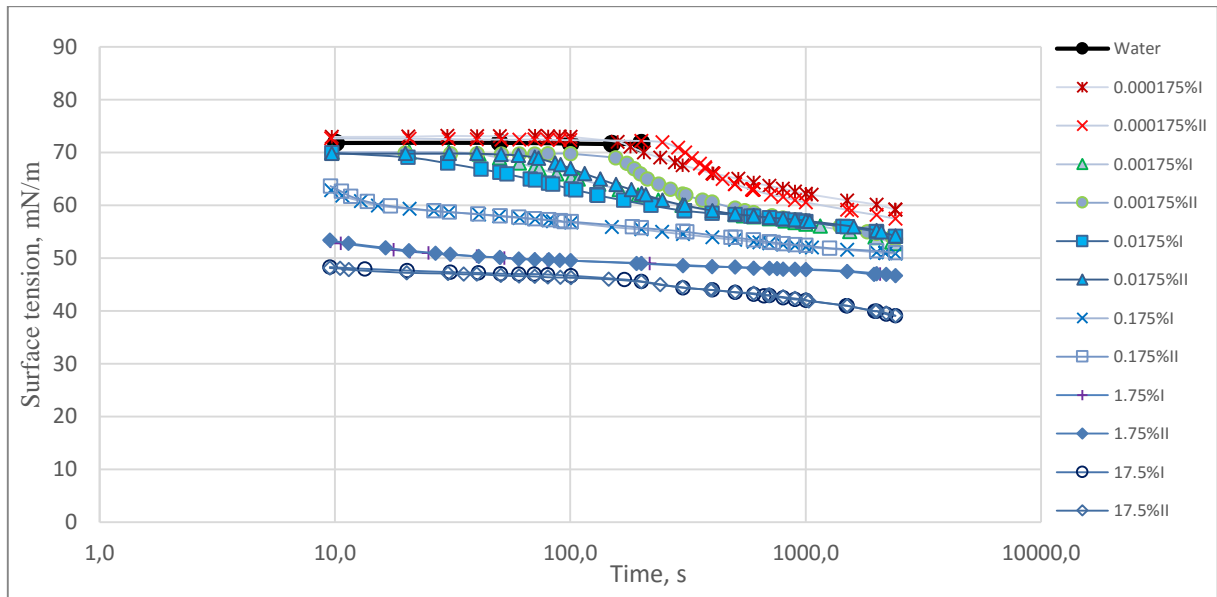
Figure 7. Surface tension as a function of time of MiSP samples (protein concentration 0.000175%-17.5%), 2400 s

Figure 7 shows the surface tension of MiSP samples (protein concentration 0.000175%-17.5%) as a function of time. In the graph, black circles with a black line corresponds to the surface tension of MilliQ water which was used to dilute the samples to obtain the desired protein concentration. It appears in the graph that the surface tension of the most diluted samples, 0.000175%I and 0.000175%II (two replicas), is around the surface tension of MilliQ water (~72 mN/m) at the beginning of the measurement. The surface tension of these samples is constant until around 300 seconds, and then it starts to decrease. The higher the protein concentration is, the lower is the surface tension at the beginning of the measurements. Also, the decline of the surface tension with time is less sharp compared to the samples containig a higher protein concentration.
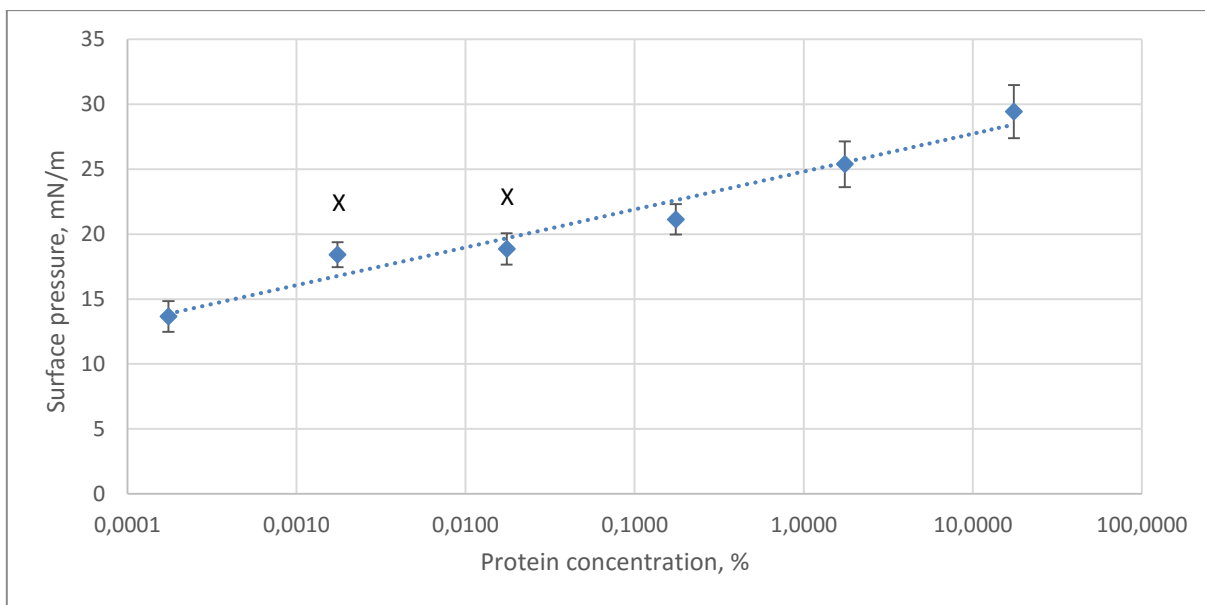
Figure 8. Surface pressure as a function of protein concentration (%) in MiSP samples after 2400 s.

Figure 8 displays the surface pressure as a function of the protein concentration (%) in MiSP samples after 2400 seconds. The graph shows that the higher the protein concentration is, the higher is the surface pressure. According to Table 9, all samples, except samples with protein concentrations 0.00175% and 0.0175% (marked with x in Figure 8), are significately different. In Figure 8, the error bars represent the standard deviation of the replicates. It can be seen that the most varying are the most concentrated samples.
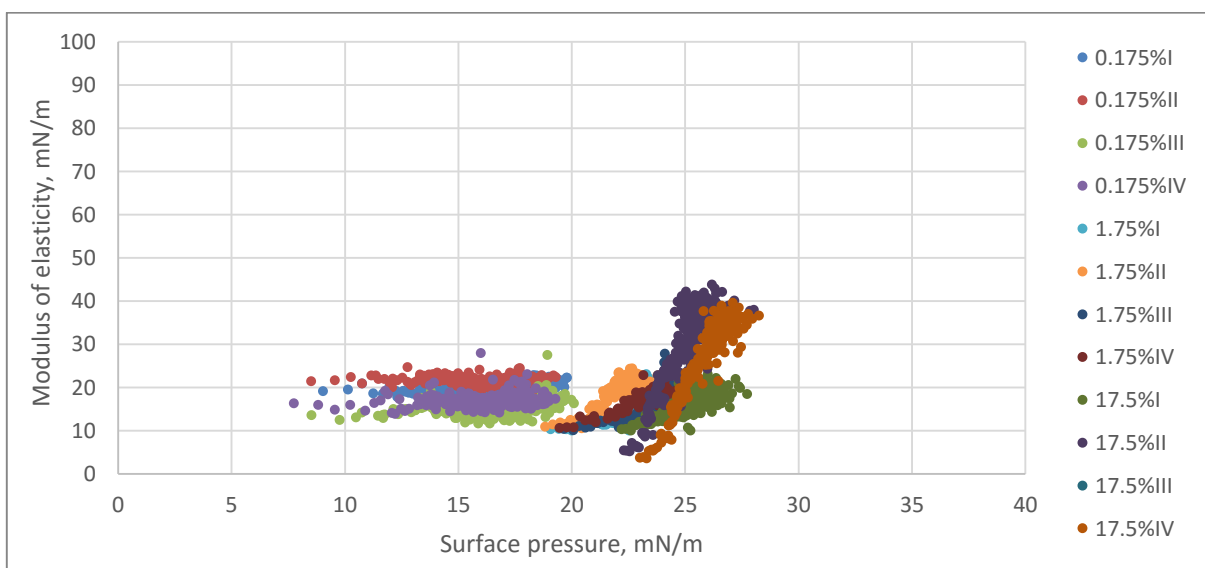
Figure 9. Modulus of elasticity as a function of surface pressure (MiSP samples).

Figure 9 shows the modulus of elasticity as a function of the surface pressure of MiSP samples (0.175%-17.5% of protein). The modulus of elasticity of the most diluted samples

changes very little during the measurement. The surface pressure of these samples is lower than the surface pressure of the other samples. For the most concentrated samples (17.5% proteins), the surface pressure does not change very much but compared to other samples there is very rapid increase in the modulus of elasticity.

Section 2: Surface tension, surface pressure and modulus of elasticity values of solutions with different lactose/MiSP ratios (with and without electrolytes).
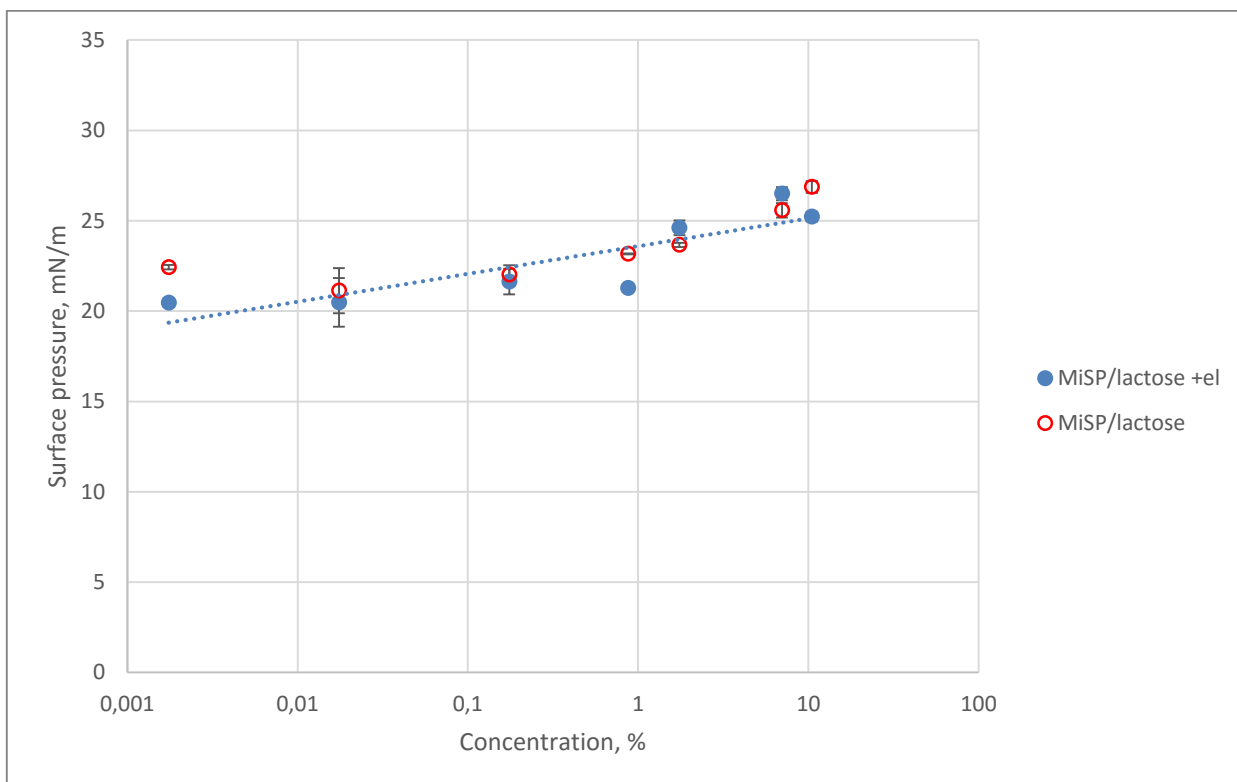
Figure 10. Surface pressure as a function of time in different MiSP/lactose ratio samples with (blue, filled circle) and without (red, hollow circle) added electrolytes after 2400 seconds.

Figure 10 shows the surface pressure of MiSP/lactose solutions with different ratios as a function of protein concentration (%) after 2400 seconds. Blue filled circles represent samples with added electrolytes and red, hollow circles, represent samples where no elctrolytes were added. The graph shows that the higher the protein content in the sample is, the higher is the surface pressure. This applies to both sample sets. The error bars, in Figure 10, represent the standard deviation. It can be seen that, in general, the standard deviation is relatively small. The most variable are samples that contained 0.0175% of proteins (MiSP/lactose ratio 0.1/99.9).
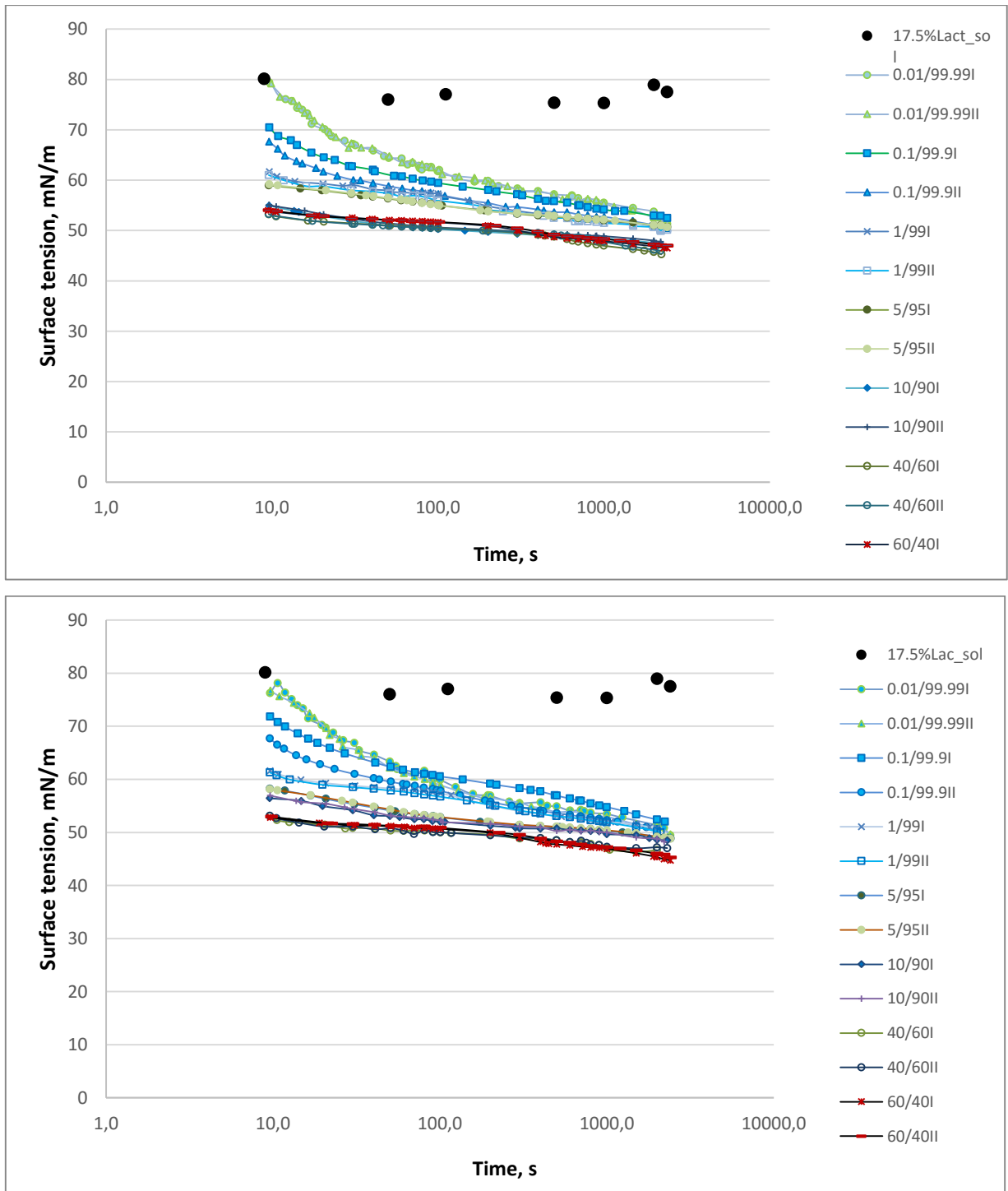
Figure 11. Surface tension as a function of time, with (upper graph) and without (lower graph) added electrolytes, measuring time 2400s.

Figure 11 displays the surface tension of MiSP/lactose solutions as a function of time. The upper graph shows the sample set with added electrolytes, and the lower graphs shows the sample set without added electrolytes. In both graphs black circles represent the surface tension of a 17.5% lactose solution. In both cases, it shows that the surface tension of the most

diluted samples (ratio 0.01/99.99) at the beginning of the measurement is around the surface tension of the 17.5% lactose solution (around 78-80 mN/m). These samples had the most prominent decrease in the surface tension during the measurement time. Mainly, the higher is the protein concentration in the solution is, the lower is the surface tension at the beginning. However, surface tension change of the samples with MiSP/lactose ratio 60/40 and 40/60 during the measurement time has no visible difference.
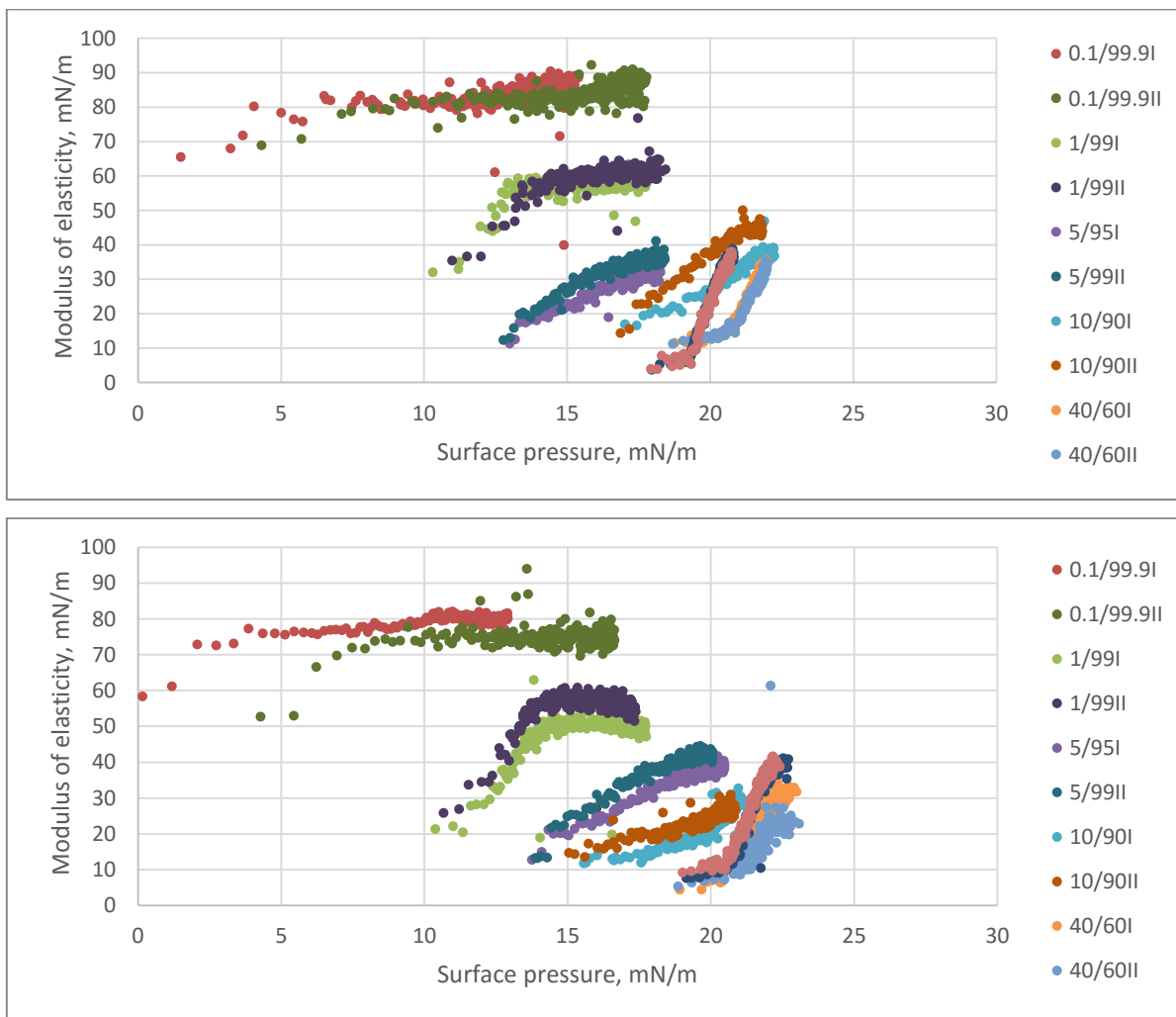
Figure 12. Modulus of elasticity as a function of surface pressure in samples with different MiSP/lactose solution with (upper graph) and without (lower graph) added electrolytes.

Figure 12 shows the modulus of elasticity as a function of surface pressure in the samples with different MiSP/lactose ratios. The upper graph shows the samples with added electrolytes can be seen, and the lower graph shows the samples without added electrolytes. The results show similar behavior of the samples depending on their MiSP/lactose ratio in both sample sets. It means that the samples 0.1/99.9 in both graphs have the highest modulus of elasticity and it remains more or less constant during the measurement. The surface

pressure of the most diluted samples are mainly lower than samples with higher protein concentrations.

Samples with a MiSP/lactose ratio of 1/99 in both graphs have also a very similar and significantely higher modulus of elasticity than samples that have a higher protein concentration. The surface pressure is, however, lower than for samples with a higher protein concentration.

Samples that have the highest protein concentration (60/40) show highest increase in modulus of elasticity during the measurement. The modulus of elasticity in both cases for ratio 60/40 in the end of the measurement (2400 s) is near 40 mN/m. The surface pressure is slightly higher for the sample that contained no added electrolytes.

Section 3: Morphology of spray dried MiSP-lactose solution particles

In Figure 13, SEM images of powder particles obtained through spray drying MiSP/lactose solutions with different ratios, is displayed. The bar corresponds to 10 µm. Letters from B to G correspond respectively to MiSP/lactose ratios from 0.1/99.99 to 60/40. Exact protein concentration in the feed of the samples is shown in Table 7.
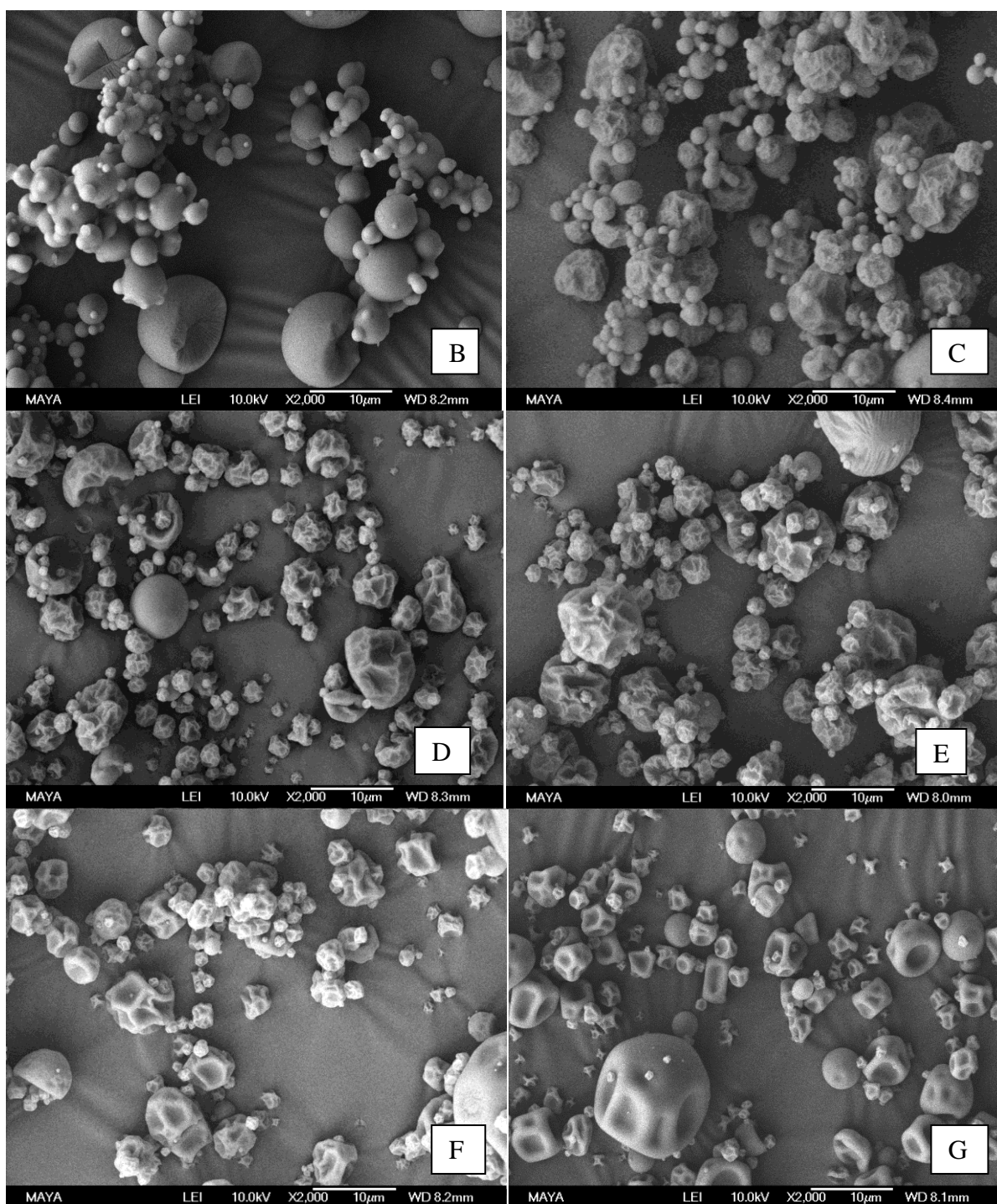
Figure 13. Scanning Electron micrographs of solution with different MiSP/lactose ratio (description: see Table 7 below).

Table 7. Explanation of the marks A-G in Figure 12 as a MiSP/lactose ratio and protein concentration (%) in corresponding samples.

Letter of Figure 12	MiSP/lactose ratio	Protein in MiSP/lactose solution (%)
A	0.01/99.99	0.00175
B	0.1/99.99	0.0175
C	1/99	0.175
D	5/95	0.875
E	10/90	1.75
F	40/60	7
G	60/40	10.5

In image B, with a MiSP/lactose ratio 0.1/99.9, the particles have predominantely a smooth surface. There are many small particles adhering to each other. Few bigger particles have a noticable signs of collapsing. Image C shows powder particles with a MiSP/lactose ratio of 1/99. These particles are far less smooth and mainly covered with small dents and ridges. Smaller particles are rather smooth and similar to particles with lower protein concentration. In Image D (MiSP/lactose ratio 5/95), the particles have larger dents and ridges compared to image C. Particles in image E (MiSP/lactose ratio 10/90) are very similar to particles in image D. In image D, some particles have a smoother surface than the powder particles in image E where all the particles are covered with dents and ridges. Image F shows particles with a MiSP/lactose ratio of 40/60. It is clearly visible that the particles have big dents and ridges on the surface. In image E (MiSP/lactose ratio 60/40), the dents are slightly larger compared to the previous sample.

5. Discussion

Section 1: The surface tension, surface pressure and modulus of elasticity of MiSP solutions with different protein concentrations.

In Figure 7 in Section 1, it appears that a higher protein concentration results in a lower surface tension. The most diluted samples (0.000175%) have a constant surface tension at the beginning of the measurements. It is around the surface tension of the pure MilliQ water which was used to dilute the samples. It is likely that at this point the surface tension is caused by the water molecules. After some time, proteins migrate to the surface and cause a decrease in surface tension. As more and more proteins cover the surface, the lower will the surface tension be. This can be seen in other samples as well. Replicates of the samples containing 0.00175% proteins have a similar behaviour. However, the surface tension is already lower at the beginning of the measurement and the decrease of the surface tension is slower. In this case there is a ground for believing that when the measurement starts, there is already some amount of proteins present on the surface. During the measurement, more and more proteins adsorb to the surface which leads to a lowering of the surface tension. In Figure 8, it possible to see that the surface rheological properties are connected to the protein concentration. This is reasonable because the higher the protein concentration is in the sample, the more likely is that some proteins are closer to the surface and due to higher amount more pressure they express on the surface of the drop after migration. In addition, it can be seen that the surface pressure of the samples after 2400 seconds is linearly dependent on the protein concentration of the samples.

Figure 9 shows the modulus of elasticity as a function of the surface pressure of the same MiSP samples. One could assume that proteins give elastic properties to the air-liquid interface. According to the results it seems that it applies to the most concentrated sample (17.5% proteins). During the measurement, the increase of the modulus of elasticity of these samples is very steep, and interestingly, it differs very much from the diluted samples. Diluted samples have a slight increase of the modulus of elasticity or it remains practically constant during the 40 minutes measurement time. This may be caused by the proteins that have, to some extent, already accumulated on the surface at the beginning of the measurement. In the solutions with a higher protein concentration, the protein layers grow thicker, and thus the increase of the modulus of elasticity, i.e. elastic properties.

Figure 14 shows the obtained surface tension values of the samples at 0.3 seconds after the formation of the droplet. Figure 7 was used as a base for the extrapolation and the exact data obtained through extrapolation is brought out in Appendix (Table 14 & 15). The surface tension values at 0.3 seconds were calculated to get an insight if some of the proteins have accumulated on the surface of the particle during spray drying. The graph shows that the most diluted samples (0.000175%) have the highest surface tension. The following two dilutions (0.00175-0.0175%) have a very similar surface tension at 0.3 seconds. Both have a slightly lower surface tension than the most diluted sample. However, the surface tension of the sample with a protein concentration of 0.175% has higher surface tension than the most diluted sample. Samples containing 1.75% and 17.5% of proteins (non-diluted concentrate) have significantly lower (see Table 9) surface tension than the other samples. The samples with a protein concentration of 17.5% have the lowest surface tension.

These results (Figure 14) imply that the samples containing 0.000175%-0.175% of proteins have no proteins or very little amount of proteins on the surface because the surface tension of the droplet is very close to the surface tension of water. Samples 1.75% and 17.5% of proteins have significantly lower surface tension than the others.
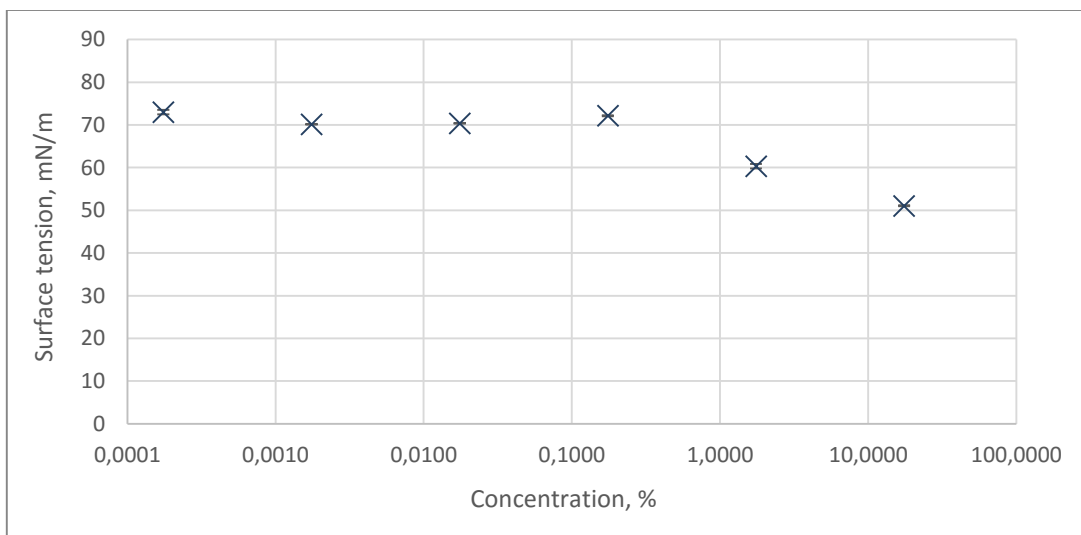
Figure 14. Surface tension of Milk Serum Protein concentrate as a function of concentration (%) predictively at 0.3 s after the drop was formed (average of 2 samples). The results are obtained through extrapolation.

Section 2: Surface tension, surface pressure and modulus of elasticity values of solutions with different lactose/MiSP ratios (with and without electrolytes).

In Section 2, solutions with different MiSP/lactose ratio were studied. Firstly, the impact of added electrolytes was evaluated. The results imply (Figure 10) that adding electolytes to the MiSP/lactose systems did not have a noticable effect. Although, according to the literature (Rabe et al., 2011), ions give the ionic strength to the solution and have a remarkably effect on the adsorption process, but the obtained results do not confirm this. However, it cannot be said that the electrolytes do not have an effect on the protein adsorption. Firstly, it is possible that the amount of electrolytes added to the solution were not significant to have an crucial effect on the results. Secondly, it is likely that the added electrolytes had a certain effect but the effect was not large enough for the equipment to detect it. It is necessary to mention, that to some extent, the inaccuracy may be caused by the different times of measurement and human factor. However, it can be said that there is no significant difference between the sample sets (table 11). Due to no significant difference, in the following discussion part, sample sets with and without electrolytes are considered as one sample set.

In Figure 11, the surface tension of MiSP/lactose samples as a function of time, is shown. As concluded earlier, there is no significant difference between samples with and without electrolytes, and this is confirmed by these graphs and statistics (for statistics, see Table 11). In both cases, the surface tension of the most diluted sample (0.01/99.99) is around the surface tension value of the 17.5% lactose solution. It may be suggested that at the beginning of the measurement proteins have not yet migrated to the surface of the droplet. After a while, proteins start to migrate to the surface and it is seen as a decrease of surface tension. As in Section 1, the higher the protein concentration in the sample, the lower the surface tension at the beginning of the measurement is, and the decrease of the surface tension seems less drastic.

The change of the surface tension of MiSP/lactose solutions, can be compared to a certain extent, to the change of the surface tension of the solutions in Section 1. Looking on the surface tension of MiSP/lactose solutions and the MiSP diluted samples that have the same protein concentration, it can be observed how lactose have an effect on the protein migration. At a first glance, it seems that when proteins are in the solution with lactose, their migration is faster. However, it may be more likely that the lactose causes the higher surface tension, and

tension, and when proteins start to migrate to the surface, the decrease of the surface tension is more noticeable.

Figure 12 shows the modulus of elasticity as a function of the surface pressure. Samples with and without added electrolytes were separately pointed out. As mentioned before, the obtained results are very similar, the electrolytes do not seem to have an effect on the surface tension and surface pressure. Instead, the surface tension and surface pressure seems to be dependent on the protein concentration. However, these results are very controversial. Samples that contained smaller amount of proteins had significantly higher modulus of elasticity compared to the samples with a higher amount of proteins. It is important to notice that the trends on the graphs, in Figure 12, are similar to the ones in Figure 9, where the modulus of elasticity of Milk Serum Protein samples (protein concentration 0.00017%-17.5%), are displayed. It means that the samples with the highest protein concentration have the most sharp and drastic increase of the modulus of elasticity during the measurement. The smaller the protein concentration is, the less sharp is the increase of modulus of elasticity during the measurement time of 40 minutes. This may imply that the presence of lactose may not be the cause of the differences in the modulus of elasticity of the diluted samples and the MiSP-lactose systems. In addition, the literature does not show any sign that lactose could increase the modulus of elasticity so drastically. There have been suggestions in the literature that the addition of lactose increases the viscosity of the solutions and thereby influence the surface properties. Even if lactose increases the viscosity, the effect cannot be so drastic on the modulus of elasticity. During the measurement period, the author of this thesis noticed that if the solution is heated above 30 °C, the modulus of elasticity decreased. In the experiments, it was necessary to increase the temperature of the samples to 30 - 40 °C to keep the lactose from precipitating. The measurements took place at 25°C, which means that during the course of time, the samples cooled down. Due to the fact that the lipids in milk have very different melting temperatures (ranges from -30 to 40 °C) (Walstra et al., 1999), it may be possible that during the measurement, some lipids solidified and caused the higher modulus of elasticity values that can be seen in the Figure 12. To confirm this theory, it is necessary to perform additional experiments, especially due to the fact that the fat concentration in the samples was exceptionally small (0.09% of fat in 100 g of MiSP concentrate). Another explanation may be that lactose crystallizes during the measurement. However, in this case, warming up the samples would not have a so remarkable effect.
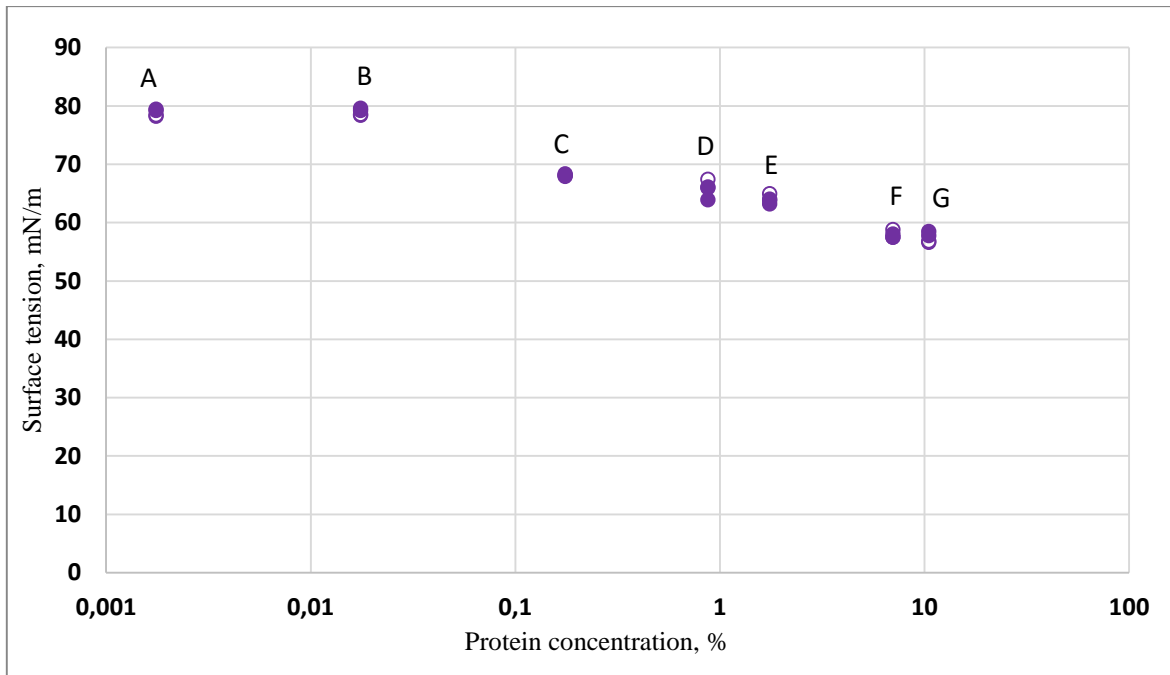
Figure 15. Surface tension of different MiSP/lactose solutions as a function of concentration, 0.3 seconds after drop formation (prediction). The letter A-G are explained in Table 7.

Figure 15 shows the surface tension of different MiSP/lactose solutions as a function of protein concentration at 0.3 seconds from the start of the drop formation. As in Section 1, this data is obtained through extrapolation and should represent the surface tension at 0.3 seconds after the drop started to form at the beginning of Pendant drop measurement. In addition, 0.3 s is approximately the lifetime of the droplet, i.e. time it takes for particle to dry during the spray drying process.

In all cases, samples with added electrolytes are marked as filled circles, and samples without added electrolytes are marked as hollow circle. Table 7 explains what the letters (A-G) in Figure 15 means, and what the MiSP/lactose ratio in the corresponding samples is. In Figure 15 it is shown that the samples that have the lowest protein concentration (A and B), have a surface tension slightly below 80 mN/m. It is around the surface tension of 17.5% lactose solution. The higher the protein concentration is, the lower the surface tension. By looking on Figure 15, it is possible to distinguish 4 main regions: A-B, C, D-E and F-G. A-B has the highest surface tension whereas F-G has the lowest. Samples D and E have also rather similar surface tension. Table 9 shows the statistical analysis done for these results. It shows that samples A and B are not significantly different from each other. Also, samples D and E are not significantly different from each other, and samples F and G are not significantly different from each other. These results indicate that spray dried particles of samples A and B have

similar morphology, spray dried particles of samples D and E have similar morphology and spray dried particles of F and G have similar morphology.

Section 3: Comparison of the surface rheology of the MiSP/lactose systems (0.3 s) with the morphology of the spray dried particles of the feed solutions.

Fäldt (1994) demonstrated that the surface of pure lactose powder particle is entirely smooth. Image B shows morphology of particles that had protein concentration of 0.0175%. These particles are almost entirely smooth. In addition, the surface tension of the droplet (ratio 0.1/99.9) at 0.3 seconds (78.95 mN/m) (Table 8) indicates that proteins have not accumulated to the surface of the droplet at this time. If the MiSP/lactose ratio changes so that the protein concentration increases, the surface of the particles become less smooth and wrinkles start to appear on the particle surface. This is visible on image C (MiSP/lactose ratio 1/99, protein concentration 0.175%). This is connected to the surface tension value (68.12 mN/m) in Table 8. If the protein concentration increases even more, bigger dents and ridges appears on the surface. In the samples with the highest protein concentrations, the dents and ridges have grown exceptionally large. In general, the appearance of the powder in the SEM images could be explained by the surface tension obtained by extrapolation, seen in Figure 14. It means that the morphology of the spray dried samples D and E are very similar to each other, and the morphology of the spray dried samples F and G are very similar to each other. It may assume that the morphology of the samples A and B should look the same.

Interestingly, in the most concentrated samples the dents are very larger. This may be an evidence of the elastic properties of the proteins on the surface of the particle. Nuzzo (2015) suggested that in her doctoral thesis as well. The Author described that the more elastic the droplet is, the more elastic is the film that forms around the droplet during drying. This in turn leads to a situation where the drop won´t break during drying, and is less susceptible to contraction when the drop starts to shrink. In the other hand, the droplet that is much less elastic have a smooth particle surface as the water has evaporated. The results obtained regarding the modulus of elasticity in this work do correspond to results from Nuzzo (2015). Before giving any final conclusion regarding this, additional experiments are needed, in order to exclude any aspects that may cause the higher modulus of elasticity in this system.

Table 8. A conclusive overview of protein content, average surface tension and spray dried particle morphology of solutions with different MiSP/lactose ratio

MiSP-lactose ratio	Protein conc., (%)	Average surface tension mN/m (0.3s)	Morphology of the particles according to SEM micrographs
0.01/99.99	0.00175	78.83	-
0.1/99.9	0.0175	78.95	Smoot particles, few large damaged particles, many small smooth particles
1/99	0.175	68.12	Many particles with ridges and dents, small particles are smooth
5/95	0.875	65.84	Almost all particles are with ridges and small dents, dents are bigger
10/90	1.75	63.99	All particles have dents and ridges, similar to 5/95, but dents have increased
40/60	7	57.95	All particles have dents and clear ridges, larger dents and ridges
60/40	10.5	57.42	All particles have dents, dents are deeper than in sample with ratio 40/60

Table 8 represents a conclusive overview of MiSP/lactose ratio, protein concentration (%), average surface tension of the droplet and the morphology of the spray dried particles. It shows that the higher the protein concentration is, the lower is the surface tension. The lower the surface tension is, the deeper are the dents and the ridges on particle surface. All in all, it can be said, that surface tension and protein concentration can be an indicator of the morphology of the spray dried particles.

Statistics

Table 9. Statistical analysis of MiSP samples and MiSP/lactose samples at 0.3 seconds after the drop formation.

Samples	Protein concentration, X/Y, %	t stat/t critical two-tail	Significant /not significant difference	Degrees of freedom (df)
MiSP solutions	0.000175/0.00175	7.51/4.30	Significant	2
	0.00175/0.0175	-3.18/4.30	**not significant**	2
	0.175/0.0175	18.48/4.30	Significant	2
	0.175/1.75	31.23/12.7	Significant	1
	1.75/17.5	24.57/4.30	Significant	2
MiSP/lactose solutions	0.00175/0.0175	-0.318/2.44	**not significant**	6
	0.0175/0.175	37.56/2.78	Significant	4
	0.175/0.875	3.11/2.44	Significant	6

	0.875/1.75	2.28/2.45	**not significant**	6
	1.75/7	12.65/2.45	Significant	6
	7/10.5	0.985/2.57	**not significant**	5

Table 10. Statistical analysis of MiSP solutions (Surface pressure as a function of concentration).

Source of Variation	SS	df	MS	F	F crit
Concentration (0.000175%-17.5%)	826.92	5	165.38	64.748	2.494
Total	913.77	39			**F>Fcrit**

Table 11. Statistical analysis of MiSP/lactose solutions (Surface pressure as a function of concentration)

Source of Variation	SS	df	MS	F	F crit
Concentration (0.00175%-10.5%)	57.370	6	9.562	12.326	4.283
With and without electrolytes	1.554	1	1.554	2.003	5.987
Error	4,654	6	0.776		
Total	63.578	13			**F>Fcrit** **F<Fcrit**

6. Environmental aspect

This thesis focused of milk serum proteins, the rheology of milk serum protein solutions, spray drying and morphology of spray dried particles. Spray drying is a quick way to produce powders, and drying in general enables the producers to economize the transportation costs. On the other hand, producing milk serum protein concentrate may rather be an energy intensive way to separate milk serum proteins compared to whey, which is a by-product in cheese production. Thus, although milk serum proteins are obtained directly from the milk and are less affected by the production process, compared to whey proteins, its production may require a bigger energy consumption.

7. Conclusions

The results in **Section 1** regarding diluted MiSP samples showed that the surface tension and the surface pressure were proportional to the protein concentration of the samples. The higher the protein concentration was, the lower was the surface tension and the higher was the surface pressure. The protein concentration showed certain connection with the modulus of elasticity as well. In these samples, it seems that a higher protein concentration resulted in a higher modulus of elasticity.

The results obtained in **Section 2,** showed that the surface pressure and the surface tension of different MiSP/lactose solutions were dependent on the protein concentration in the solutions. Similarly to MiSP samples in Section 1, samples with higher protein concentration in the MiSP/lactose solutions had a lower surface tension and a higher surface pressure.

Results obtained regarding the modulus of elasticity in **Section 2** were controvery. The results showed mainly that the lower the protein concentration was, the higher was the modulus of elasticity. This is very different from results published in different scientific articles. It is likely that these modulus of elasticty values obtained in Section 2 are not related to proteins, nor lactose. To observe this phenomenon and exclude other factors, it is necessary to conduct additional experiments.

The results obtained in **Section 3** showed a correlation between the protein concentration, surface tension at 0.3 seconds, and the morphology of corresponding spray dried particles. In the samples where the surface tension, after 0.3 seconds, was near to the surface tension of the pure lactose solution, the spray dried particles were mostly smooth. The more proteins the feed contained, the less smooth and more dented was the surface of the particles. This corresponded to the surface tension and the surface rheology of the samples. The more protein the samples contained, the lower was the surface tension and the higher was the surface pressure of the samples. The samples that contained the higest amount of proteins resulted in spray dried particles with large dents and ridges.

8. Future recommendations

Based on the results obtained in this work, the Author has 3 main recommendations for subsequent experiments.

The most controversial results were obtained with the measurements of the modulus of elasticity. Thus, it is recommendable to increase the measurement temperature to observe, if elevated temperature affects the measurements. If the results are remarkably lower, then it is very likely that the milk lipids play important part in the modulus of elasticity measurements. If not, then is is possible to exclude the theory and focus on other aspects that my affect the results.

The second recommendation involves milk serum proteins diluted solutions and scanning electron microscope images. Spary drying diluted MiSP samples and obtaining SEM images would be very useful for comparing the morphology of these particles with morphology of spray dried MiSP/lactose samples.

The third recommendation refers to the used technique in the surface rheology measurements, the Pendant drop method. According to Saad et al (2011), the pendant drop method is not the most accurate method for measuring surface tension and it has certain disadvantages. In the future it would be recommended to repeat some of the experiments with another method to compare the obtained data in this study.

9. SUMMARY

The aim of this master's thesis was to evaluate rheological properties of diluted milk serum protein systems, and observe how rheological properties are affected by diluting the system with lactose solution in various ratios. Furthermore, the relationship between the rheological properties of the milk serum protein-lactose system, and the morphology of the spray dried particles, was evaluated. In addition, another objective was to evaluate whether the electrolyte concentration in milk serum protein system has an effect on rheological properties of milk serum protein solutions.

In the current work rheological properties of diluted milk serum protein samples and milk serum protein-lactose systems (with and without electrolytes) were evaluated using Pendant drop and oscillating Pendant drop method. Furthermore, different milk serum protein-lactose systems were spray dried and the morphology of the particles was captured by scanning electron microscope.

The results showed that adding electrolytes did not affect the surface tension and surface pressure of MiSP-lactose system significantly. There was certain difference between water and lactose solutions when they were used as a matrix for the milk serum proteins. However, it was not possible, apart from increasing the surface tension of the general solution, to ascertain how exactly lactose affects the protein adsorption to the surface.

A certain connection between surface tension, surface pressure and the spray dried particle morphology was found. Samples that had the highest surface tension at 0.3 second had almost a smooth particle surface. The lower the surface tension and higher the surface pressure were, the deeper dents and larger ridges did the spray dried particles tend to have. It showed that the protein concentration had the main impact on the morphology of the spray dried particles.

KOKKUVÕTE

Antud magistritöö eesmärk oli hinnata piima seerumi valkude lahuseid ja piima seerumi valkude-laktoosi süsteemi pinna reoloogiat. Lisaks hinnati piima seerum valkude-laktoosi süsteemi reoloogiliste omaduste sõltuvust pihustuskuivatatud osakeste morfoloogiast ning elektrolüütide mõju süsteemile.

Eksperimentaalses osas kasutati piima seerumi valkude lahuste ja piima seerumi valkude-laktoosi süsteemi reoloogia uurimiseks rippuva tilga (*Pendant drop method*) ja ostsilleeruva rippuva tilga (*Oscillating Pendant drop*) meetodit. Piima seerumi valkude-laktoosi süsteemide edasiseks uurimiseks need pihustuskuivatati ja pulbriosakeste morfoloogia jäädvustati Skaneeriva elektronmikroskoobiga.

Käesoleva magistritöö tulemused näitasid, et elektrolüütide lisamisel ei olnud piima seerumi valkude-laktoosi süsteemile märkmisväärset mõju. Laktoosi mõju hindamine antud süsteemile näitas, et teatud erinevus on olemas võrreldes proovidega, mis ei sisaldanud lisatud laktoosi. Samas on oluline teha lisakatseid ja välja selgitada, kuidas mõjutab laktoos maatriksina piima seerum valkude adsorptsiooni.

Tulemustest selgus lisaks, et proovi pindpinevuse ja proovi pihustuskuivatatud osakeste morfoloogia vahel on seos. Proovidel, millel oli 0,3 sekundit pärast tilga moodustumist pindpinevus kõige kõrgem, oli pihustuskuivatatud osakesed enamjaolt siledad. Mida madalam oli proovidel pindpinevus, seda enam esines pihustuskuivatatud osakeste pinnal mõlke ja volte. Proovidel, millel oli kõige kõrgem valgusisaldus, olid pinnal kõige sügavamad mõlgid. See näitas, et piima seerumi valkude-laktoosi süsteemis on valkudel peamine mõju pihustuskuivatatud osakeste morfoloogia kujunemisele.

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Table 12. Setup parameters of program Windrop (Tracker™)

Parameter	
Top parameters:	Surface tension and temperature
Bottom parameters:	Angle, tension/temperature
Drop density (kg/dm^3):	0.997040[1]
Bulk density (kg/dm^3):	0.00117[2]
Drop status:	Pendant
Threshold:	100
Calculation mode:	L/R precise
Initial volume:	8 µ after 2 drop
Injected volume:	Around 5 µl
Stop on time after (s):	300 (for water); 2400 s (for sample)
Expel:	2 drops
Syringe	4
Sampling parameters	
Sampling mode:	Fixed steps
Sampling, s	0
Time 1:	150 s
End sampling, s	2 s.
Time 2:	180 s
Temp:	On
Saving:	Data Syncronize

Table 13. Measurement parameters of Tracker™ for oscillating Pendant drop experiments.

Parameter	Used value
Amplitude:	2
Period:	3
Shift:	0
Active cycles:	6
Blank cycles:	8
Oscillation sampling, s:	0.3
Volume profile:	Sinusoidal profile
Start regulation after, s:	10

[1] Density of water at 25ºC (Haynes, 2015-2016)
[2] Density of air at 25ºC (Haynes, 2015-2016)

Table 14. Results from extrapolation of surface tension of MiSP samples, 0.3 seconds (0.000175%-0.0175%)

0.000175%		0.000175%		0.00175%		0.00175%		0.0175%		0.0175%	
Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m
0.1	72.54	0.1	73.59	0.1	70.12	0.1	70.3	0.1	70.42	0.1	70.42
0.2	72.6	0.2	73.46	0.2	70.12	0.2	70.23	0.2	70.41	0.2	70.35
0.3	72.63	0.3	73.39	0.3	70.12	0.3	70.2	0.3	70.41	0.3	70.31
1	72.74	1	73.17	1	70.11	1	70.09	1	70.4	1	70.19
2	72.8	2	73.05	2	70.11	2	70.02	2	70.37	2	70.12
3	72.83	3	72.97	3	70.11	3	69.98	3	70.35	3	70.07
4	72.86	4	72.92	4	70.11	4	69.96	4	70.33	4	70.04
5	72.87	5	72.88	5	70.11	5	69.94	5	70.31	5	70.02
6	72.89	6	72.85	6	70.11	6	69.92	6	70.29	6	70
7	72.9	7	72.82	7	70.11	7	69.91	7	70.27	7	69.99
8	72.91	8	72.8	8	70.11	8	69.89	8	70.25	8	69.97
9	72.92	9	72.78	9	70.11	9	69.88	9	70.23	9	69.96
9.7	72.96	9.6	72.67	9.7	70.05	9.7	69.82	9.71	69.96	9.68	69.8
10.8	72.94	11.0	72.77	11.1	70.16	10.8	69.89	10.84	69.89	10.89	70.07
11.9	72.98	12.1	72.79	12.3	70.06	11.9	69.88	12.02	69.88	12.01	69.89
13.1	72.88	13.3	72.68	13.5	70.01	12.99	69.84	13.29	69.87	13.08	70.09

Table 15. Results from extrapolation of surface tension of MiSP samples, 0.3 seconds (0.175%-1.75%)

0.175%		0.175%		1.75%		1.75%		17.5%		17.5%	
Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m	Time, s	Surface tension, mN/m
0.1	90.52	0.1	97.3	0.1	62.21	0.1	63.1	0.1	52.07	0.1	51.92
0.2	86.28	0.2	92.15	0.2	60.79	0.2	61.58	0.2	51.48	0.2	51.32
0.3	83.81	0.3	89.14	0.3	59.96	0.3	60.7	0.3	51.14	0.3	50.98
1	76.45	1	80.21	1	57.5	1	58.08	1	50.13	1	49.95
2	72.22	2	75.06	2	56.09	2	56.56	2	49.55	2	49.36
3	69.74	3	72.05	3	55.26	3	55.68	3	49.21	3	49.01
4	67.98	4	69.92	4	54.67	4	55.05	4	48.97	4	48.77
5	66.62	5	68.26	5	54.21	5	54.57	5	48.78	5	48.58
6	65.51	6	66.91	6	53.84	6	54.17	6	48.63	6	48.42
7	64.56	7	65.76	7	53.53	7	53.83	7	48.5	7	48.29
8	63.75	8	64.77	8	53.25	8	53.54	8	48.38	8	48.18
9	63.03	9	63.9	9	53.01	9	53.29	9	48.29	9	48.08
9.6	62.85	9.6	63.66	9.6	53.15	9.5	53.36	9.5	48.28	9.5	48.14
10.8	61.80	10.6	62.63	10.6	52.81	10.5	53.05	10.5	48.2	10.5	48.04
11.9	61.13	11.7	61.71	11.7	52.45	11.4	52.78	11.5	48.07	11.5	47.82
12.9	60.77	12.8	61.19	12.7	52.32	12.4	52.58	12.4	48.03	12.4	47.79

Table 16. Example of the surface tension of MilliQ water during calibration, 200 seconds.

Time	Surface tension, mN/m
0,051	71,858
10,08	71,823
50,02	71,852
100,12	71,708
150,06	71,619
200,00	71,672
200,13	71,828

Table 17. The surface tension of 17.5% lactose solution, 2400 seconds.

Time	Surface tension, mN/m
0,052	78,479
8,96	80,139
50,04	76,035
111,30	77,036
500,68	75,398
1000,07	75,316
2000,69	78,929
2399,88	77,510