Impact of the complex interplay of formulation components on stability of spray dried emulsions

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La vie n'est facile pour aucun de nous. Mais quoi, il faut avoir de la persévérance, et surtout de la confiance en soi. Il faut croire que l'on est doué pour quelque chose, et que, cette chose, il faut l'atteindre coûte que coûte.

Marie Curie (Physicist)

Zusammenfassung

Sprühgetrocknete Emulsionen wie bspw. Säuglingsanfangsnahrung oder Kaffeeweißer sind häufig verwendete Systeme in der Lebensmittelindustrie. Die Formulierung der sprühgetrockneten Emulsionen besteht meist aus einem Matrixmaterial aus Stärkeabbauprodukten, einer Öl-Phase und aus emulgierenden Bestandteilen wie Proteinen und niedermolekularen Emulgatoren. Diese Emulsionen werden mittels Emulgierung, Zerstäubung und dem Trocknungsvorgang in die Pulverform überführt, um eine hohe Stabilität der Produkte über einen langen Lagerzeitraum zu ermöglichen. Die Stabilität der sprühgetrockneten Emulsionen wird durch Prozess und Formulierung beeinflusst. Die Formulierungsbestandteile lenken die Stabilität während aller Prozessschritte im flüssigen Zustand vor dem Übergang in die Pulverform oder während der Lagerung der Pulver. Der erstere Fall wird vom Verhalten der emulgierenden Bestandteile bestimmt wobei im letzteren Fall alle Formulierungsbestandteile einen Einfluss haben. Das Verhalten der emulgierenden Bestandteile steht im Zusammenhang zu ihrem Grenzflächenverhalten. Die physikochemischen Mechanismen während der Lagerung basieren neben anderen Effekten auf dem Phasenübergangsverhalten der Öl-Phase. Beide Fälle werden durch die komplexen Wechselwirkungen der Formulierungsbestandteile beeinflusst.

Dieser Zusammenhang wurde im Detail untersucht. Die Ergebnisse zeigen, dass das Verhalten der emulgierenden Bestandteile durch die komplexen Wechselwirkungen der Formulierungsbestandteile an der Grenzfläche, in der wässrigen und Öl-Phase verändert wird. So beeinflusst das veränderte Verhalten der emulgierenden Bestandteile die Stabilität der sprühgetrockneten Emulsionen in Öltropfengröße und Verkapselungseffizienz während der Prozessschritte im flüssigen Zustand. Die physikochemischen Mechanismen während der Pulverlagerung werden hingegen durch komplexe Interaktionen der niedermolekularen Emulgatoren und Öl-Phase beeinflusst. So wurden sprühgetrocknete Emulsionen mit kristallisierten niedermolekularen Emulgatoren an der O/W Grenzfläche durch Freisetzung des verkapselten Öles destabilisiert. Da die beschriebenen Effekte von der molekularen Struktur der Formulierungsbestandteile abhängen, kann eine gezielte Variation der Formulierungsbestandteile zur Erhöhung der Stabilität sprühgetrockneter Emulsionen führen.

Abstract

Spray dried emulsions like infant formulae or coffee creamer are commonly applied systems in the food industry. The formulation of spray dried emulsions consists of a matrix material like starch conversion products, an oil phase, and emulsifying constituents like proteins and low molecular weight emulsifiers. These emulsions are transferred into powder form via emulsification, atomization and a drying step to maintain a high stability of these products over a long storage period. The stability of spray dried emulsions is affected by processing and formulation. The formulation components guide the stability during each processing step in the liquid state before powder transformation or during the storage of powders. The former case is determined by the performance of emulsifying constituents, as the latter case is determined by all formulation components. The performance of emulsifying constituents during processing steps in the liquid state is linked to their interfacial behaviour. The physicochemical mechanisms during storage are based on phase transition behaviour of the oil phase beside other phenomena. Both cases are further influenced by the complex interplay of formulation components.

This interrelation was investigated in detail. The results indicate that the performance of emulsifying constituents changes due to the complex interplay of formulation components at interface, in water and oil phase. The altered performance of emulsifying constituents affects the stability of spray dried emulsions in oil droplet size and encapsulation efficiency during processing steps in the liquid state. The physicochemical mechanisms, however, are influenced by complex interactions of low molecular weight emulsifiers and oil phase. Spray dried emulsions were destabilized by crystallized low molecular weight emulsifiers at the o/w interface, releasing encapsulated oil. Since all described effects strongly depend on the molecular structure of formulation components, a tailored variation in formulation components may increase the stability of spray dried emulsions.

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List of abbreviations

Abbreviation	Meaning
90H	Phosphatidylcholine of soy origin with saturated fatty acids
CIC	Critical interfacial concentration
CIELAB	Colour space with vectors (L*, a* and b*)
CMC	Critical micelle concentration
DE	Dextrose equivalent
DMPE	Dimyristoylphosphatidylethanolamine
DMSO	Dimethylsulfoxid
GS 37	Glucosesyrup with dextrose equivalent 37
HLB	Hydrophilic-lipophilic balance
IFT	Interfacial tension
LMWE	Low molecular weight emulsifier
MCT-oil	Middle-chain-triglyceride oil
MD 14 and 9	Maltodextrin with dextrose equivalent 9 and 14
MoDi	Mono- and diglyceride
NMR	Nuclear magnetic resonance
ODSD	Oil droplet size distribution
O/W	Oil/water interface
PSD	Powder size distribution
S100	Phosphatidylcholine of soy origin with unsaturated fatty acids
SANS	Small angle neutron scattering
SAXS	Small angle X-ray scattering
SDSD	Spray droplet size distribution
SEC-MALS-DRI	Size exclusion chromatography – multi angle light scattering – differential refractive index
SEM	Scanning electron microscopy
WPI	Whey protein isolate
XRPD	X-ray powder diffraction
β-LG	β-lactoglobulin

a	Capillary radius
A	Area
$\Delta A/A_0$	Change in area during dilatational rheology
Bo	Bond number
Bou	Boussinesq number
Ca	Capillary number
Dn	Needle diameter
E*	Complex dilatational modulus
E‘	Elastic dilatational modulus
E‘‘	Viscous dilatational modulus
g	Gravity constant
G*	Complex interfacial shear modulus
G‘	Elastic interfacial shear modulus
G‘‘	Viscous interfacial shear modulus
R_0	Radius curvature at drop apex
R	Measurement cell radius
r	Cylindrical drop coordinate
s	Length
ΔV	Amplitude of volume oscillation
V_d	Droplet volume
V_{max}	Maximal droplet volume
Wo	Worthington number
z	Cylindrical drop coordinate
η	Interfacial viscosity
η_o & $\eta_{p/c}$	Dynamic viscosity of oil & protein/starch conversion product solution
$\Delta \mu$	Viscosity difference between water and oil phase
$\Delta \rho$	Density difference between water and oil phase
σ and Υ	Interfacial tension
σ_0	Interfacial tension of water
φ	Tangent angle
ω	Oscillation frequency
ϕ	Phase angle

I. Motivation and objectives

Spray drying is widely applied in the food industry to dry and preserve liquid or paste-like products (Cuq et al., 2011; Santos et al., 2018), producing powders with free flowing particles (Santos et al., 2018). Common spray dried systems are pickering emulsions (Mwangi et al., 2020; Yan et al., 2020), but most frequently oil in water emulsions (Delshadi et al., 2020; Geranpour et al., 2020). Application areas of emulsions are dairy goods, infant formula, powdered beverages and toppings or creamers. These emulsions are formulated and emulsified in the liquid state, are subsequently atomized, dried in a spray tower with hot air (Bakry et al., 2016) and are often stored for several years (Walstra et al., 2006). The formulation of these spray dried emulsions commonly consists of a dispersed oil phase, emulsifying constituents like proteins and low molecular weight emulsifiers (LMWEs), and a carbohydrate-based bulk material like lactose and/or starch conversion products.

The stability of spray dried emulsions is crucial and essential (Bakry et al., 2016; Cuq et al., 2011; Vega & Roos, 2006), whereby a stable spray dried emulsion is characterized by free flowing particles without lumps and a sufficiently incorporated dispersed oil phase to ensure oxidative stability (Vega & Roos, 2006). However, stability reducing mechanisms may arise either from processing factors or formulation: On the processing side, most relevant factors comprise emulsification (Håkansson, 2016; Håkansson et al., 2009, 2010, 2011, 2013; Håkansson & Hounslow, 2013; Santana et al., 2013), spray drying (Cal & Solohub, 2010; McCarthy et al., 2015; Ziaee et al., 2019), including atomization (Lefebvre & Mcdonell, 2017; Munoz-Ibanez et al., 2015; O'Sullivan et al., 2019) and drying step, affecting the particle structure (de Souza Lima et al., 2020; Schutyser et al., 2012).

With respect to the formulation, the stability of spray dried emulsions is affected by the performance of emulsifying constituents during processing steps in the liquid state before powder formation (emulsification, atomization, and drying step) and by the physicochemical mechanisms during powder storage (see **Figure I-1** at page 3). In the liquid state, the performance of the emulsifying constituents is directly correlated with the characteristics of the dispersed oil phase (as indicated in **Figure I-1**) which ideally leads (McClements & Gumus, 2016) to a stable spray dried emulsion with high encapsulation efficiency and a narrow oil droplet size distribution with small oil droplets (Vega & Roos, 2006; Vignolles et al., 2007). In the liquid state prior to spray-drying,

the emulsifying constituents stabilize the oil water interface of all oil droplets (McClements & Gumus, 2016). The mechanisms in the liquid state strongly depend on the interfacial stabilization mechanism and the resulting interfacial properties of the emulsifying constituents. Generally, all emulsifying constituents act by reducing the interfacial tension at the oil/water interface (Wilde et al., 2004). However, proteins and LMWEs differ in the mode of interfacial stabilization (as indicated in **Figure I-1**). Proteins form a viscoelastic interfacial film with intermolecular cross-linking (Dickinson, 2011; Wilde et al., 2004). In contrast, LMWEs form fluid, close-packed films at the interface. These films are stabilized with weak electrostatic interactions or Gibbs-Marangoni mechanism as restoring force to area changes in the film (Wilde et al., 2004). The interfacial performance of highly interfacial active LMWEs may lead to a stable spray dried emulsion with a small oil droplet size (Talón et al., 2019; Wilde et al., 2004) and high encapsulation efficiency; as well as the viscoelastic interfacial film of proteins may lead to a stable spray dried emulsion via prevention of coalescence of oil droplets during each processing step (Vega & Roos, 2006).

Furthermore, the formulation may affect the stability of spray dried emulsions via physicochemical mechanisms during storage time. The matrix material (also called carbohydrate based bulk material) may reduce the stability through physical mechanisms like caking or crystallization as well as chemical phenomena like Maillard reaction (Aalaei et al., 2019; Roos, 2010; Troise & Fogliano, 2013) (as indicated in **Figure I-1**). An oil phase may reduce the stability through oxidation of polyunsaturated fatty acids or other easily oxidizable constituents and any physical changes during storage affecting the encapsulation efficiency (Bakry et al., 2016; Vega & Roos, 2006; Vignolles et al., 2007). The physical changes of the oil phase may be linked to phase transition behaviour which causes a reduction in encapsulation efficiency with a release of encapsulated oil (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003) (as indicated in **Figure I-1**). These changes have been more pronounced for oil phases with saturated fatty acids than for oil phases with unsaturated fatty acids (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003).

Recent publications suggest that numerous interactions of formulation components affect the performance of emulsifying constituents during processing steps in the liquid state and potentially the physicochemical mechanisms during storage time. These publications show that the performance of emulsifying constituents is affected by

molecular structure dependent interactions of emulsifying constituents with each other (1) (Danviriyakul et al., 2002; Drapala et al., 2017; McClements & Mahdi Jafari, 2018; Shujie Wang et al., 2017; Zou & Akoh, 2013), with the carbohydrate-based bulk material in the water phase (2) (Antipova & Semenova, 1997; Baeza et al., 2004), and by interactions and phase transition behaviour of emulsifying constituents with the oil phase (3) (Hildebrandt et al., 2016; Rodríguez Patino, Rodríguez Nino, et al., 2001).

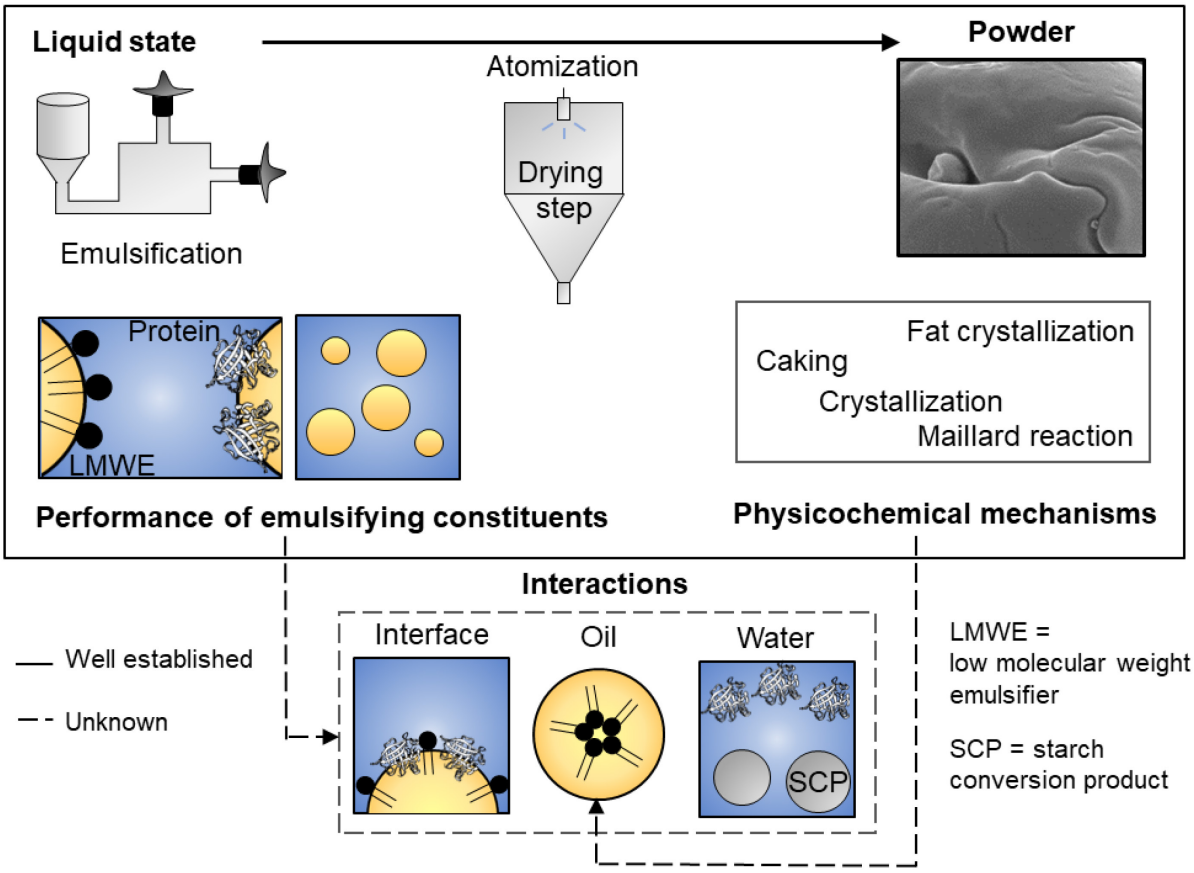

Figure I-1: Overview of the impact of the interplay of formulation and processing on the stability of spray dried emulsions.

These three effects are pictured in **Figure I-1** and represent recent objectives of research. For all three, general interactions are well known: between LMWEs and proteins (McClements & Mahdi Jafari, 2018) (1), between proteins and neutral carbohydrates like starch conversion products (Grinberg & Tolstoguzov, 1997) (2), and between oil phase and LMWEs (Garti & Yano, 2001) (3). These interactions further affect the interfacial properties: (1) LMWEs and proteins may interact at the interface via electrostatic effects, hydrophobic effects and hydrogen bonds altering interfacial properties (Murray & Dickinson, 1996). (2) Proteins and neutral carbohydrates may exhibit thermodynamic incompatibility (Grinberg & Tolstoguzov, 1997), leading to

improved interfacial properties like a decrease in interfacial tension (Antipova & Semenova, 1997) and an increase in film viscoelasticity (Baeza et al., 2004). (3) Oil phase and LMWE may interact strongly with increasing similarity in molecular structure (Garti & Yano, 2001), reducing the interfacial occupation (Hildebrandt et al., 2016). Further, interactions of oil phase and LMWE may be related to phase transition behaviour and crystallization events depending on temperature and molecular structure (McClements, 2012; Ribeiro et al., 2015), inducing a rigid interfacial behaviour (Rodríguez Patino, Rodríguez Nino, et al., 2001) and physicochemical mechanisms of the oil phase during storage time. These physicochemical mechanisms may lead to coalescence or potentially oil release in emulsions or spray dried emulsions (Fäldt & Bergenståhl, 1995; Fredrick et al., 2013; Goibier et al., 2017; Millqvist-Fureby, 2003). However, a few correlations could not be explained, yet. The impact of the aforementioned three interactions on the interfacial properties, thus on the performance of emulsifying constituents during processing in liquid state, and on the properties of spray dried emulsions during storage is not elaborated for different molecular structures of formulation components (Danviriyakul et al., 2002; Drapala et al., 2017; Fäldt & Bergenståhl, 1995; Masum et al., 2019; Millqvist-Fureby, 2003; Shujie Wang et al., 2017; Zou & Akoh, 2013).

In particular, three aspects could not be explained, yet: (1) The impact of combinations of a protein, and LMWEs of different subcategories on interfacial and resulting emulsion or spray dried emulsion properties alongside the processing steps in the liquid state. (2) The impact of the molecular structure of neutral carbohydrates like starch conversion products in application-oriented concentrations on interfacial properties of a protein at the oil/water interface. (3) The impact of the molecular structure and interactions of oil phases and LMWEs on interfacial properties of a protein at the oil/water interface. The impact of interactions and phase transition behaviour of oil phase and LMWEs on the characteristics of spray dried emulsions during storage time. These three aspects picture the complex interplay of formulation components.

Therefore, an approach is missing to understand the impact of this complex interplay of formulation components on the stability of spray dried emulsions. In detail, the impact of the interactions of formulation components on the performance of emulsifying constituents during processing steps (emulsification, atomization and drying step) and on the physicochemical mechanisms during storage time needs to be investigated. To approach this overall goal, three research objectives with corresponding expectations and general approach are defined.

Objective 1: Examine the impact of interactions of emulsifying constituents on their performance during processing

This objective considers the performance of emulsifying constituents during the processing steps: *emulsification, atomization and especially the drying step*. The performance of emulsifying constituents may be linked to interfacial properties recorded in exemplary and modelled liquid systems. More specifically, the interfacial properties may be linked to changes in the oil droplet size. However, objective 1 focuses mainly on the molecular structure dependent interactions of LMWE and protein at the interface and is affected by interactions with the carbohydrate based bulk material in the water phase, and with the oil phase. As emulsifying constituents' whey protein and its main component β-lactoglobulin are chosen as protein source and the three subcategories -lecithin, mono-and diglyceride and citrem- are chosen as LMWEs. It is expected that a combination of a highly interfacial active LMWE and whey protein may lead to a stable spray dried emulsion, since a highly interfacial active LMWE may reduce the oil droplet size (Talón et al., 2019; Wilde et al., 2004), and a viscoelastic interfacial film of proteins prevents oil droplets from coalescing during each processing step (Vega & Roos, 2006).

Objective 2: Examine the impact of interactions of formulation components in the water phase on the performance of emulsifying constituents

Objective 1 provides a base to understand the performance of emulsifying constituents during processing steps. It models the performance with interfacial analysis in liquid systems. Subsequently, objective 2 systemizes the impact of the interactions in the water phase on the performance of emulsifying constituents, which is modelled with interfacial analysi*s* as well. Interfacial analysis shall be performed with β-lactoglobulin

as model protein and main component of whey protein, and with a molecular variation of starch conversion products.

In the water phase, interactions of proteins with starch conversion products may occur and affect the interfacial properties of proteins. It is expected that addition of starch conversion products with application-oriented concentrations and varying molecular weight decreases the interfacial tension and increases the viscoelasticity for β-lactoglobulin stabilized interfaces. Such a viscoelastic interfacial film should lead to a stable spray dried emulsion, since it was reported that a viscoelastic protein film prevents oil droplets from coalescing during each processing step (Vega & Roos, 2006).

Objective 3: Examine the impact of interactions of formulation components in the oil phase on the performance of emulsifying constituents and on physicochemical mechanisms during storage

In the first instance, the systemized and modelled knowledge of objective 2 with focus on the water phase shall be extended to the oil phase. Thereby, objective 3 addresses the interactions of specific LMWEs and oil phases and evaluates their effect on the performance of combined emulsifying constituents, using interfacial analysis. Moreover, the effect of the interactions of LMWEs and oil phase on physicochemical mechanisms during storage shall be considered.

In the oil phase, interactions with LMWEs depend on molecular structure and affect the interfacial properties. It is expected that a lack in interactions between oil phase and LMWE may cause a LMWE enrichment close to the interface. That might decrease the interfacial tension and increase the interfacial reactivity, thus, elasticity to area changes for protein and LMWE stabilized systems. Such a viscoelastic interfacial film should lead to a stable spray dried emulsion, as reported for viscoelastic protein films in spray dried emulsions (Vega & Roos, 2006).

The interfacial behaviour of LMWEs further depends on their phase transition behaviour. It is expected that a protein and LMWE stabilized interface with crystallized LMWE may show a rigid behaviour. Such a rigid behaviour could be linked to crystallization based destabilization mechanisms like coalescence or potentially oil release in emulsions or spray dried emulsions (Fäldt & Bergenståhl, 1995; Fredrick et al., 2013; Goibier et al., 2017; Millqvist-Fureby, 2003). It is expected that a LMWE with

a high crystallization tendency may cause crystallization-based destabilization mechanisms like oil release in spray dried emulsions.

These specific research objectives were addressed by combining the results from the following publications, which are included in this dissertation as manuscript 1 to 4.

- **Manuscript 1:**
  Heiden-Hecht, T., Taboada, M., Brückner-Gühmann, M., Karbstein, H. P., Gaukel, V. and Drusch, S. (2021). Towards an improved understanding of spray-dried emulsions: impact of the emulsifying constituent combination on characteristics and storage stability. International dairy journal, 105134. doi: 10.1016/j.idairyj.2021.105134.

  Addresses objective 1 by characterising the impact of interactions of emulsifying constituents on their performance during processing steps. Contributes to objective 2 and 3 by examining the interfacial performance of proteins and LMWE affected by interactions in the oil and water phase. Addresses objective 3 by discussing the physicochemical mechanisms during storage.

- **Manuscript 2:**
  Taboada, M., Heiden-Hecht, T., Brückner-Gühmann, M., Karbstein, H. P., Drusch, S. and Gaukel, V. (2021). Spray drying of emulsions: influence of the emulsifier system on changes in oil droplet size during the drying step. Journal of Food Processing and Perservation, 45, e15753. doi: 10.1111/jfpp.15753

  Addresses objective 1 by characterising the impact of interactions of emulsifying constituents on their performance during processing steps.

- **Manuscript 3:**
  Heiden-Hecht, T., Ulbrich, M., Drusch, S., Brückner-Gühmann, M. (2021). Interfacial properties of β-lactoglobulin at the oil/water interface: influence of starch conversion products with varying dextrose equivalents. Food Biophysics, 16, 169-180. doi: 10.1007/s11483-020-09658-4

  Addresses objective 2 by focusing on the impact of interactions in the water phase on the performance of emulsifying constituents.

- **Manuscript 4:**
  Heiden-Hecht, T. and Drusch, S. (2021). Impact of saturation of fatty acids of phospholipids and oil phase on properties of β-lactoglobulin at the oil/water interface. Food Biophysics.

  Addresses objective 3 by focusing on the impact of interactions in the oil phase on the performance of emulsifying constituents.

II. Literature review

This literature review describes the impact of the complex interplay of formulation components on the stability of spray dried emulsions. The complex interplay of formulation components affects the performance of emulsifying constituents during processing steps before powder formation and the physicochemical mechanism during storage time. The issue will be described stepwise, starting with the impact of interactions of formulation components *at the interface, in the water and in the oil phase* on the performance of emulsifying constituents, as it is the most crucial and most frequently occurring point of this thesis. Subsequently, the impact of the complex interplay on physicochemical mechanisms will be discussed. Therefore, this section forms the basis to answer the specific research objectives 1 to 3.

II.1 Impact of interactions between proteins and LMWEs on their performance

In general, proteins and LMWEs are used as emulsifying constituents in spray dried emulsions. Proteins stabilize the o/w interface in emulsions via migration from the aqueous bulk phase, adsorption at the interface and conformational reorganization, leading to a viscoelastic interfacial film (Dickinson, 2011; Wilde et al., 2004). In contrast, LMWEs begin the interfacial stabilization process with transport from the aqueous or oil phase to the interface (Wilde et al., 2004), depending on their solvent phase or hydrophilic-hydrophobic-balance (HLB) (McClements & Mahdi Jafari, 2018; Pasquali et al., 2009). Proteins are known to stabilize emulsions with their viscoelastic interfacial film, which prevents the oil droplets from coalescing (Vega & Roos, 2006). LMWEs are known to stabilize emulsions with their high interfacial activity leading to a small oil droplet size (Talón et al., 2019). Both emulsifying constituents ideally maintain the emulsion stability against destabilization mechanisms like creaming, sedimentation, flocculation, coalescence, Ostwald ripening and phase inversion (Hu et al., 2017). Both emulsifying constituents stabilize emulsions during each processing step in the liquid state before powder formation and ideally lead to a small oil droplet size and a high encapsulation efficiency. However, the mechanisms depend on the molecular structure of the emulsifying constituents.

Common emulsifying constituents in spray dried emulsions are milk proteins (Tavares et al., 2014), phospholipids, citric acid esters of mono-/diglycerides and mono-/diglycerides (European Communities, 2016). There are two distinct structural groups

of milk proteins: the globular and compact structured whey proteins like β-lactoglobulin and the flexible random coil structured caseins. Beside the molecular structure, the interfacial stabilization of proteins is influenced by the specific isoelectric point and the pH of the surrounding medium and thus the electrostatic charging (Lam & Nickerson, 2013).

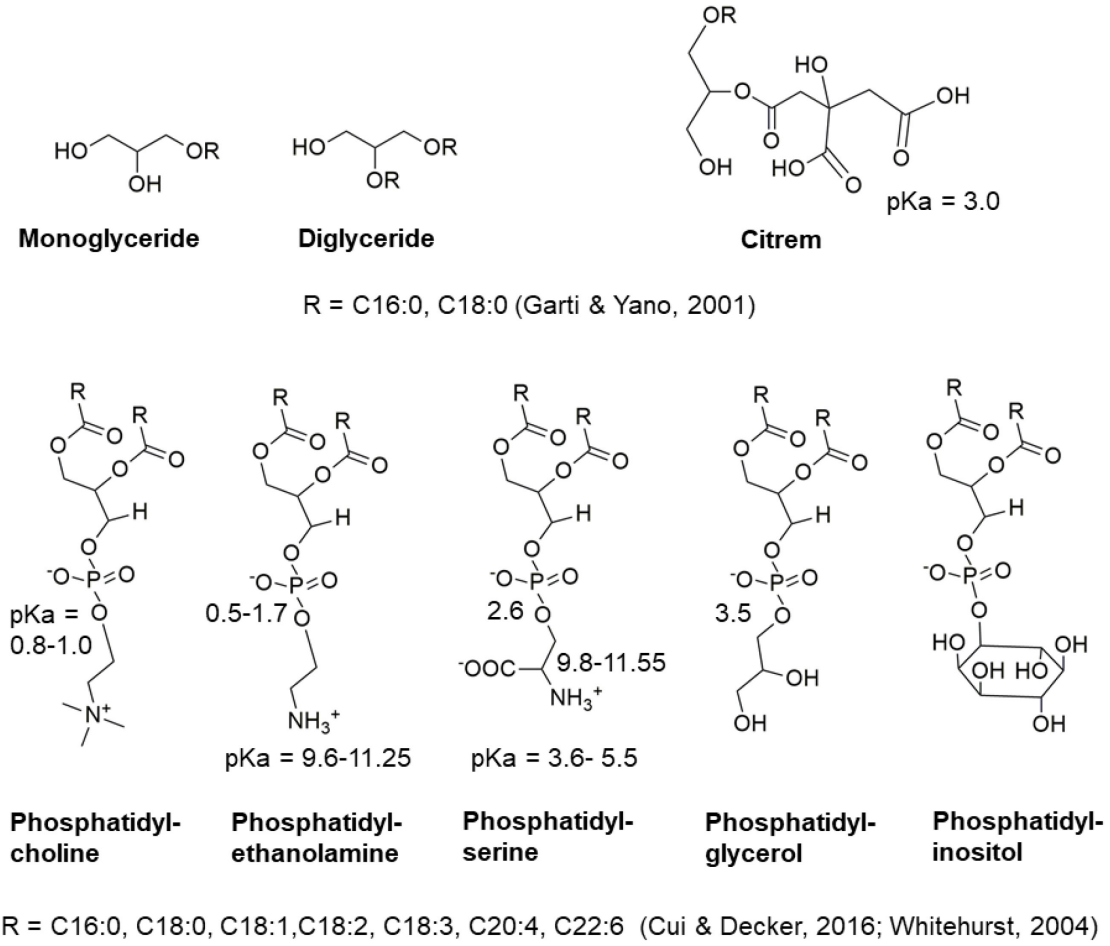

Figure II-1: Molecular structure of LMWEs - phospholipids, citrem and mono- and diglyceride according to (Cui & Decker, 2016; Garti & Yano, 2001; Ghazvini et al., 2018; Whitehurst, 2004).

In contrast, LMWEs vary in their head group and fatty acid composition (**Figure II-1**), depending on origin, extraction and modification (Arranz & Corredig, 2017; Cui & Decker, 2016; Garti & Yano, 2001; Joshi et al., 2006; Sprick et al., 2019; Van Nieuwenhuyzen & Tomás, 2008; Whitehurst, 2004). Common head groups of phospholipids are phosphatidylcholine, -ethanolamine, -serine, -glycerol and -inositol. The head groups of mono- and diglycerides are one to two hydroxyl groups (Garti & Yano, 2001; Whitehurst, 2004). For citrem as citric acid ester of mono- and diglycerides, the head groups vary in the amount of esterified citric acid, while one esterification possibility is shown in **Figure II-1** (Whitehurst, 2004). For all three LMWE

groups, the most common fatty acids and pKa values (Cui & Decker, 2016; Ghazvini et al., 2018) are summarized in **Figure II-1**. The latter determines the electrostatic charging of molecules depending on pH. This results in zwitterionic phosphatidylcholine and -ethanolamine in a pH range of 1 to approx. 11 and in anionic citrem, phosphatidylserine and -glycerol above a pH of 3.

Physical phenomena based on the net charge of emulsifying constituents are well established. Electrostatic repulsion between oil droplets increases the emulsion stability while electrostatic attraction leads to destabilization effects like flocculation (Hu et al., 2017). In contrast - at the interface, numerous attractive interactions of molecules in interfacial films lead to a high viscoelasticity, which stabilizes oil droplets against mechanical stress and coalescence (Murray & Dickinson, 1996). The viscoelasticity of the film is a result of electrostatic effects, hydrophobic effects and hydrogen bonds (Murray & Dickinson, 1996).

In a mixed interfacial film of protein and LMWE, the interactions are more complex and diverse as well as not fully understood. A single protein covers a monolayer at the interface at its critical interfacial concentration (CIC) (Schestkowa et al., 2020; Tamm et al., 2012), as a LMWE does at the critical micelle concentration (CMC) (Jahan et al., 2020). Depending on the concentration ratio, proteins are often displaced by highly interfacial active LMWE (Wilde et al., 2004). However, proteins and LMWE may also coexist (Rodríguez Patino et al., 2007) in domains or in alternating order at the interface (McClements & Mahdi Jafari, 2018) or may form complexes (Dan et al., 2013; Kotsmar et al., 2009; McClements & Mahdi Jafari, 2018). The interfacial tension and viscoelasticity of a mixed interfacial film may be reduced (Murray & Dickinson, 1996). Attractive interactions like electrostatic effects or hydrogen bonds may increase the viscoelasticity (Murray & Dickinson, 1996).

The combination of emulsifying constituents further affects the characteristics and stability of spray dried emulsions as presented in several studies (Danviriyakul et al., 2002; Drapala et al., 2017; Shujie Wang et al., 2017; Zou & Akoh, 2013). Adding lecithin to a sodium caseinate or whey protein stabilized spray dried emulsion led to a reduced oil droplet size or increased encapsulation efficiency (Danviriyakul et al., 2002; Shujie Wang et al., 2017) in comparison to adding monoglyceride (Danviriyakul et al., 2002). Potential explanations for these and other scenarios will be provided in the following chapters, considering the impact of the complex interplay of formulation

components in the water and oil phase on the performance of emulsifying constituents. These chapters explain the basics of the interactions of formulation components in the water and oil phase, illustrate their impact on interfacial properties and conclude the impact on the performance of emulsifying constituents during processing steps in liquid state.

II.2 Impact of interactions of formulation components in the water phase on the performance of emulsifying constituents

Proteins and other matrix constituents like starch conversion products are either solubilized in the aqueous phase prior to emulsification, or the carbohydrate source is added stepwise after emulsification. Presence of matrix constituents with different molecular weight may result in distinct phenomena based on the thermodynamics of polymers in solution. These comprise (I) co-solubility, (II) phase segregation or (III) complexation (de Kruif & Tuinier, 2001; Semenova, 2007). Complexation is reflected in an associative behaviour between molecules (de Kruif & Tuinier, 2001), while phase segregation results in two phases enriched in one of the molecule species due to an excluded volume effect. Therefore, the molecule species are likely not to interact with each other (de Kruif & Tuinier, 2001). All thermodynamic mechanisms depend on concentration, ionic strength, pH, isoelectric point, structure of the protein (Grinberg & Tolstoguzov, 1997) and molecular size of polysaccharides. High molecular weight, non-ionic polysaccharides and proteins are more prone to phase segregation according to the Flory Huggins Theory. This is based on the increasing probability of phase segregation with increasing molecular weight and size difference of polymers (Semenova & Dickinson, 2010). In contrast, a mixture of low molecular weight, non-ionic polysaccharides and proteins exhibits more diverse phenomena of interactions (Shukla et al., 2011). Mono- and disaccharides are known to act as conformational stabilizers for proteins, based on steric exclusion to the protein and cohesive forces of mono- and disaccharides beside other phenomena (Shukla et al., 2011).

Thermodynamic effects of proteins and carbohydrates or carbohydrate-based polymers in the aqueous phase affect the composition and properties of interfacial films. Most of the earlier performed studies investigated air/water interfaces (Antipova & Semenova, 1997; Baeza et al., 2004, 2005; Perez et al., 2010; Ruíz-Henestrosa et al., 2008). Since the interactions between proteins and neutral polysaccharides are taking place in the water phase, we assume a general transferability to o/w interfaces.

Antipova and Semenova observed that the presence of a low molecular weight, non-ionic polysaccharide like glucose with low concentrations increases the interfacial tension of a protein stabilized interface. The authors ascribed their observation to an increased thermodynamic affinity of the protein to water, correlating with the presence of low molecular weight, non-ionic polysaccharides, as proved by multi angle light scattering (Antipova & Semenova, 1997). In contrast, presence of high molecular weight, non-ionic polysaccharides like maltodextrin and dextran causes different effects on interfacial properties of proteins. Since addition of high molecular weight, non-ionic polysaccharides to a protein in the water phase increases the viscosity (Dokic et al., 1998), the adsorption of the protein may be decelerated according to the Stokes Einstein equation as part of the Ward Tordai theory (Ward & Tordai, 1946). The interfacial tension may be reduced by the excluded volume effect, as described in the case of broad bean (Vicia faba) (Antipova & Semenova, 1997). This phenomenon was correlated to a protein enrichment at the interface (Rodríguez Patino & Pilosof, 2011), which caused an increased viscoelasticity for a β-lactoglobulin film surrounded with non-ionic xanthan in the water phase (Baeza et al., 2004; Perez et al., 2010). The described effects on the interfacial properties are summarized in **Figure II-2**.

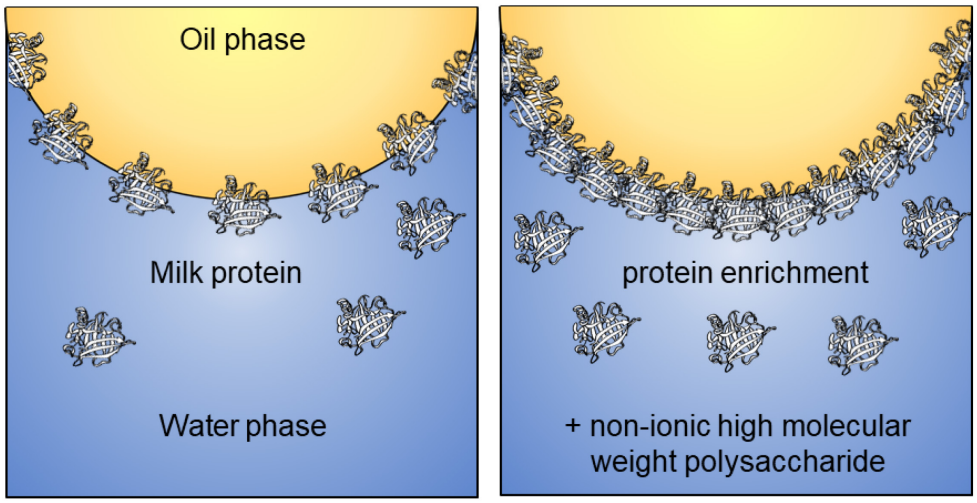

Figure II-2: Interfacial properties of exemplified milk protein films at o/w interface with presence of a non-ionic high molecular weight polysaccharide.

All of the above-mentioned studies have been performed in dilute systems, and, so far, the impact of thermodynamic effects on interfacial properties of proteins has not been investigated at high dry matter content as it occurs in emulsions for spray-drying. Since thermodynamic effects depend on concentration (Grinberg & Tolstoguzov, 1997), this

will be of interest for interfacial and emulsion research. In addition, an increase in dry matter content changes the characteristics of samples (Dokic et al., 1998) and might affect applicability of methods for interfacial characterization and accuracy of results. The increase in dry matter content changes the flow behaviour and viscosity of samples (Dokic et al., 1998), which influences interfacial results and methods. Bertsch and Fischer (2019, 2020) and Bertsch et al. (2018, 2020) recently investigated the impact of charged anisotropic nanocrystals at the air/water interface (Bertsch et al., 2018, 2020; Bertsch & Fischer, 2019, 2020). In these studies, the increased bulk viscosity had an impact on the applicability of interfacial methods. General operating windows and methodology prerequisites for interfacial methods are outlined in the following publications (Berry et al., 2015; Freer et al., 2005; Ravera et al., 2010; Renggli et al., 2020; Tajuelo & Rubio, 2018). A thematically appropriate consideration of instrumental and methodological limitations is presented and discussed in a recent publication which focuses on the impact of the interactions between β-lactoglobulin as whey protein and starch conversion products as non-ionic polysaccharide or also called neutral polysaccharide or neutral carbohydrate (Heiden-Hecht, Ulbrich, et al., 2021).

In general, the interactions of neutral carbohydrates like starch conversion products and proteins show a high potential to improve the stability of spray dried emulsions. This is concluded since the addition of starch conversion products caused an increase in interfacial viscoelasticity (Baeza et al., 2004) and a viscoelastic interfacial protein film is generally known to prevent oil droplets from coalescing during each processing step in liquid state (Vega & Roos, 2006).

II.3 Impact of interactions of formulation components in the oil phase on the performance of emulsifying constituents

In contrast to proteins and polysaccharides, LMWEs may be solubilized in the oil phase, and the fatty acid chains of LMWEs may interact with the oil. The interactions between LMWEs and oil phases are increasing with increasing similarity in fatty acid composition and, thus, solubility (Garti & Yano, 2001). These interactions are based on dispersion forces and weak π-interactions (Belitz et al., 2009; Walstra, 2003) and are influencing the interfacial adsorption, interfacial arrangement, interfacial tension and film characteristics. Recent publications show that the adsorption at the interface was affected for phospholipids strongly interacting with the oil phase. This leads to an

increase in concentration required to reach a monolayer of phospholipids (Hildebrandt et al., 2016). The interfacial arrangement for intermediate soluble, saturated phospholipids is a highly condensed monolayer (Hildebrandt et al., 2016). An oil phase with saturated fatty acids causes a multilayer arrangement with high density for any phospholipid at the o/w interface (Hildebrandt et al., 2016) (see **Figure II-3**). The effect of phospholipid and oil phase interactions on interfacial tension and viscoelasticity of phospholipid and β-lactoglobulin stabilized systems has been discussed in a recent publication (Heiden-Hecht & Drusch, 2021). According to the presented results, we assume a general impact of LMWE and oil phase interactions and phase transition behaviour on interfacial properties of LMWE and protein stabilized systems which needs to be further confirmed.

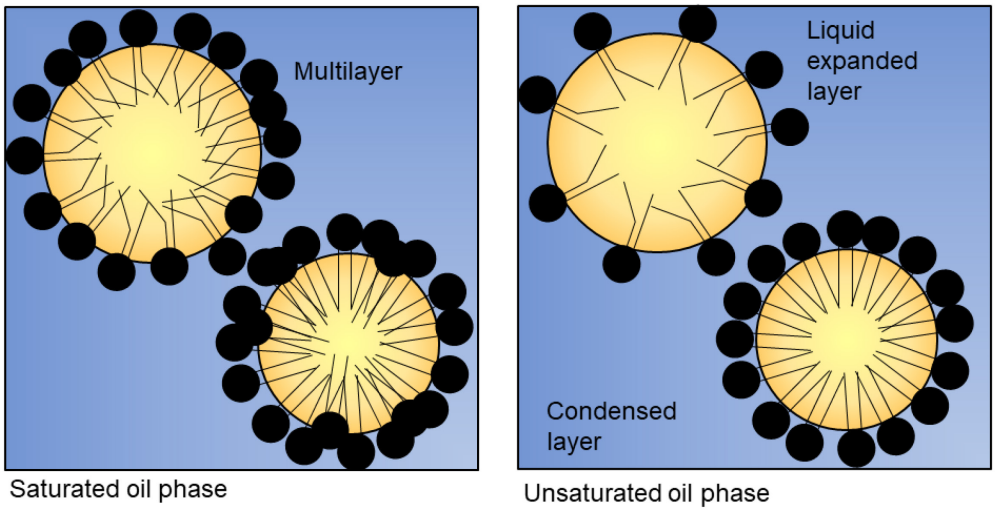

Figure II-3: Interfacial characteristics, depending on saturation of LMWEs and oil phase according to Hildebrandt et al (2016).

The phase transition behaviour of LMWEs depends on their fatty acid composition and the ambient temperature. LMWEs tend to crystallize at the interface or tend to act as crystallized emulsifiers (McClements, 2012; Ribeiro et al., 2015) which are inducing a higher reduction in interfacial tension (Krog & Larsson, 1992) and a rigid interfacial behaviour (Rodríguez Patino, Navarro García, et al., 2001). The viscoelasticity of a LMWE and protein stabilized film may even increase by crystallization events of LMWE as described for a/w interfaces (Golding & Sein, 2004; Sánchez & Rodríguez Patino, 2004). Detailed knowledge about the interfacial organization of crystallized or liquid LMWE with proteins at o/w interfaces may be gained with new emerging techniques like X-ray and neutron scattering, grazing incidence scattering, interfacial

microrheology and single particle Brownian dynamics (Ghazvini et al., 2015; Gilbert, 2019; Jaksch et al., 2019; Oberdisse & Hellweg, 2017; Park et al., 2016), which lays the foundation for an upcoming research field with high potential.

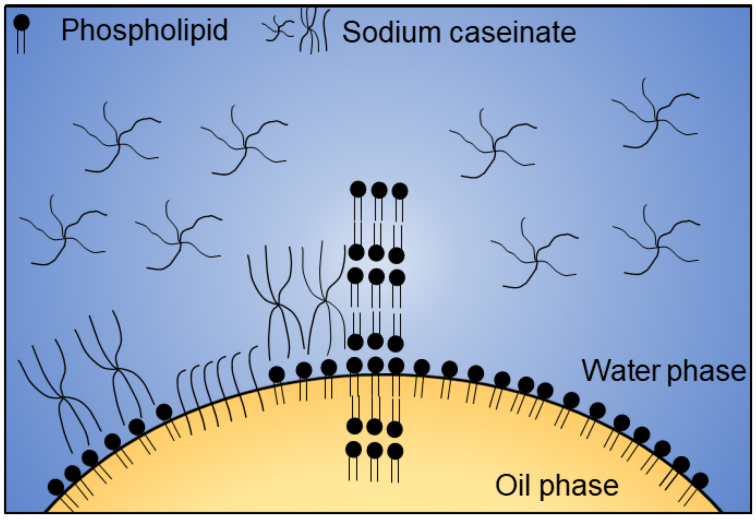

Figure II-4: Mechanistic model of interfacial film stabilized with sodium caseinate and phospholipid (Yesiltas et al., 2019).

Small angle X-ray scattering and neutron scattering are used to visualize the organization of interfacial films. A recent publication presented a model of an interfacial film with sodium caseinate and a phospholipid (Yesiltas et al., 2019). The proteins and phospholipids overlapped in multilayer structures, as illustrated in **Figure II-4** (Yesiltas et al., 2019). Another study presented results of phospholipid monolayers at the air/water interface, investigated via neutron scattering. The results were fitted to a model, which proved the applicability to investigate the organization and orientation of fatty acid chains in monolayers. Beside the organization of interfacial films, this method may be used to identify interactions of LMWE, depending on ionic strength, pH, temperature and interfacial tension (Campbell et al., 2018).

In comparison, grazing incidence X-ray diffraction, interfacial microrheology and single particle Brownian dynamics are methods to investigate the organization of fatty acid chains of LMWE in interfacial films. Details about the phase state, the polymorphic form and the orientation of solid LMWEs at the interface can be identified (Di Cola et al., 2017; Ghazvini et al., 2015; Juárez et al., 2018; Stefaniu & Brezesinski, 2014; Vollhardt & Brezesinski, 2015). A recent study investigated the surface rheology for Dilauroyl- and Dimyristoylphosphatidylethanolamine (DMPE) in a Langmuir trog setup with microrheology. It was shown that the surface elasticity increased in the solid phase

state for DMPE, caused by the change in organization of fatty acid chains (Ghazvini et al., 2015). Another promising method to broaden the comprehension of the solid character of monolayers is single particle Brownian dynamics with a combination of optical microscopy and cyclic voltammetry (Juárez et al., 2018).

In general, the presented interrelation of interactions between oil phase and LMWE, phase transition behaviour of LMWE and resulting interfacial properties of protein and LMWE stabilized interfaces are very complex and not nearly systemized, so far. However, the interrelations offer a tremendous potential to adjust the performance of emulsifying constituents by tailoring the fatty acid composition of LMWE and oil phase.

II.4 Impact of the complex interplay of formulation components on physico-chemical mechanisms during storage

The previous chapters described the impact of the complex interplay of formulation components on the performance of emulsifying constituents in detail. To complete the explanations of the impact of the complex interplay of formulation components on the stability of spray dried emulsions, the physicochemical mechanisms during storage time are outlined as well.

During storage of spray dried emulsions, the complex interplay of formulation components affects the stability via physicochemical mechanisms. Some of these physicochemical mechanisms like caking or Maillard reaction of the matrix material (Roos, 2010; Troise & Fogliano, 2013) or crystallization effects of the oil phase are well established (Fäldt & Bergenståhl, 1995; E. H. J. Kim et al., 2005; Millqvist-Fureby, 2003). The crystallization effects in the oil phase may be affected by interactions with LMWE. A recent and outstanding publication showed that LMWE induced crystallization events in emulsions may influence the shape of oil droplets (Denkov et al., 2015). Under certain cooling circumstances, the oil drop may undergo a transformation in shape of polyhedron, hexagonal prism and other shapes to a rod-like asperity as shown in **Figure II-5** (at page 17). This change in drop shape is based on phase transition in interfacial films at a temperature close to the melting temperature of the dispersed phase. The drop deformation occurs, if the interfacial tension decreases to 4 or 8 mN/m and if the interfacial film creates a sufficiently high bending moment to curve the drop shape. This phenomenon was observed for triacylglyceride and LMWE combinations under specific conditions, namely: small oil droplets, low cooling rates, LMWE with saturated long fatty acid chains and small head groups

(Cholakova et al., 2016, 2019; Denkov et al., 2015, 2019; Guttman et al., 2017). However, the relevance for spray dried emulsions needs to be elaborated to define and delimit the impact of this effect on powdered systems. It may be assumed that these crystallization events start in liquid emulsions and affect characteristics of spray dried emulsions during storage time.

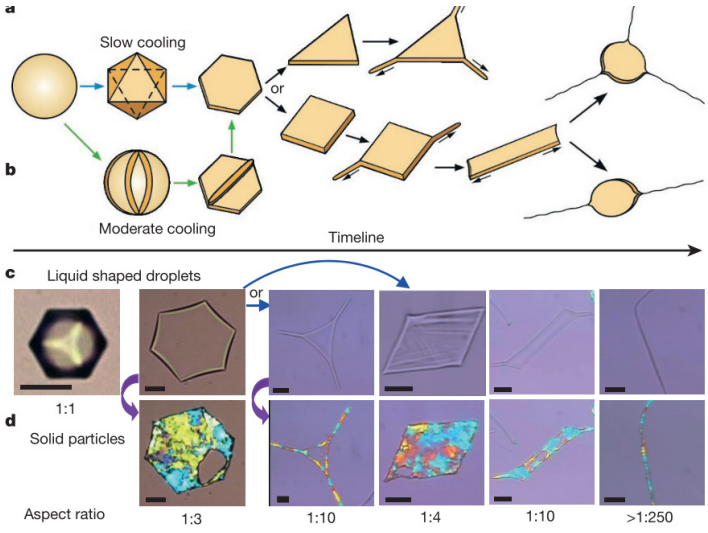

Figure II-5: Oil droplet shape deformation in emulsions by crystallization phenomena at the interface (Denkov et al., 2015).

Under usual conditions, LMWE induced crystallization events may cause heterogenous nucleation with a templating function (Douaire et al., 2014; McClements, 2012; Ribeiro et al., 2015). This template may inhibit oil droplet coalescence acting as a crystallization barrier (Fredrick et al., 2013; Goibier et al., 2017), or may induce oil droplet coalescence by interfacial damage (Fredrick et al., 2013; Goibier et al., 2017). Furthermore, the interfacial damage may be linked to a change of crystal shape by polymorphic transition. Polymorphic transition from α to β crystals shows a change in crystal form from spherical to needle like shape, affected by temperature and time (Awad et al., 2008; McClements, 2012). In a spray dried emulsion, the interfacial damage was often induced by fat crystallization (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003) from oil phases with saturated fatty acids instead of oil phases with unsaturated fatty acids (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003). In general, the impact of LMWEs on oil phase transition is barely understood. A recent publication discussed the stability of spray dried emulsions stabilized with proteins and LMWEs. The previously described effects influenced the stability of these spray dried emulsions (Heiden-Hecht, Taboada, et al., 2021).

III. Manuscript 1

Towards an improved understanding of spray-dried emulsions: Impact of the emulsifying constituent combination on characteristics and storage stability

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Abstract

In spray-dried emulsions a wide range of emulsifying constituents including proteins and low molecular weight emulsifiers are used. Due to their different behaviour, combinations of different emulsifying constituents are common, whereupon their interactions may also adversely affect powder properties and stability. Therefore, the impact of whey protein isolate alone or in combination with lecithin, mono-/diglyceride and citrem as low molecular weight emulsifiers on powder characteristics and storage stability were investigated. Temperature stresses were applied to induce instability phenomena. A specific combination of protein and low molecular weight emulsifiers resulted in a reduction in oil droplet size while maintaining encapsulation efficiency. Induction of crystallization through low temperature stress induced oil release in samples, in which templating for heterogeneous nucleation took place. High temperature stress caused Maillard reaction, protein-fat complexation and phase transition of the matrix resulting in colour changes and reduction of extractable oil.

Keywords: phase transition, crystallization, dairy powder, interface, protein, emulsifier, quality, encapsulation

III.1 Introduction

Spray-dried emulsions like infant formula, spray-dried aroma compounds or coffee creamer are widely present in the food sector. One aim of the spray-drying is to maintain a high quality over a long time of storage and thus physical and chemical stability of spray-dried emulsions is of utmost importance and a key aim (Cuq et al., 2011). The stability of spray-dried emulsions is determined by the particle characteristics, which in turn depend on process parameters (Håkansson et al., 2009; McCarthy et al., 2015; O'Sullivan et al., 2019; Taboada et al., 2019, 2020) and formulation (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003; Roos, 2010; Troise & Fogliano, 2013; Vega & Roos, 2006; Vignolles et al., 2007). The formulation of spray-dried emulsions is composed of a matrix material e.g., starch conversion products, an oil phase and an emulsifying constituent like milk proteins and/or low molecular weight emulsifiers. The stability may be affected by undesired physical or chemical phenomena associated with glass transition, caking or Maillard reaction of the matrix material (Roos, 2010; Troise & Fogliano, 2013). With respect to the oil phase key determinants for the stability are a high encapsulation efficiency, a low extractable oil content and a small oil droplet size (Vega & Roos, 2006; Vignolles et al., 2007). As recently reviewed, all these parameters depend on the adsorption behaviour of the emulsifying constituents and the stability of interfacial film formed by these constituents (Ravera et al., 2020; Zhou et al., 2020).

Different emulsifying constituents – like the above-mentioned proteins and low molecular weight emulsifiers – show a different behaviour during interfacial adsorption and resulting film characteristics. Low molecular weight emulsifiers frequently show a higher interfacial activity and tend to displace proteins from the interface (Bos & van Vliet, 2001; Wilde et al., 2004). Furthermore, their high interfacial activity often leads to a smaller oil drop size in emulsions (Talón et al., 2019) and stabilizes oil droplets after breakup upon mechanical stress like it occurs e.g., during atomization. In contrast, proteins usually form a viscoelastic film at the interface which acts as physical barrier against coalescence (Murray & Dickinson, 1996; Wilde et al., 2004; Zhou et al., 2020) and preserves the oil droplet size and encapsulation efficiency of spray-dried emulsions during particle formation (Vega & Roos, 2006).

As a consequence, proteins and low molecular weight emulsifiers are frequently co-formulated and coexist in emulsions. The emulsifying constituents may also interact

with each other and coexist at the interface with resulting change in interfacial behaviour. These interactions are based on hydrogen bonding, hydrophobic and electrostatic effects (Dan et al., 2013; Kotsmar et al., 2009). Hydrophobic effects occur between the hydrophobic tail of the low molecular weight emulsifier and the hydrophobic core of the protein (Dan et al., 2013; Kotsmar et al., 2009) while electrostatic effects are based on the net charge of proteins and low molecular weight emulsifiers in dependence on the isoelectric point (Lam & Nickerson, 2013) or pk_a value (Cui & Decker, 2016; Whitehurst, 2004) respectively. A low viscoelasticity is a result of less, repulsive or hydrophobic interactions (Murray & Dickinson, 1996; Wilde et al., 2004). A higher viscoelasticity is based on more attractive interactions (Dan et al., 2013; Kotsmar et al., 2009).

Furthermore, interactions of emulsifying constituents with the oil phase gain importance with respect to phase transition phenomena during storage of liquid and spray-dried emulsions. Phase transition in the form of crystallization of the lipophilic constituents may result in a reduction of the powder stability by oil release. This process is temperature-dependent (Awad et al., 2008; Boode et al., 1991; Tippetts & Martini, 2009) and is affected by the emulsifying constituent combination. Depending on the fatty acid composition and thus, solubility and crystallization temperature, low molecular weight emulsifiers may act as template for nucleation and may protect the oil droplet against oil release during phase transition (Garti & Yano, 2001).

It is obvious that the interactions of formulation components and effects on physicochemical mechanisms will affect the stability of the spray-dried emulsions during storage. Different studies on the impact of the composition of the matrix material (Masum et al., 2019), the oil phase (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003) or the emulsifying constituents combination (Drapala et al., 2017; Zou & Akoh, 2013) on powder stability exist. For emulsifying constituents, interactions between the three commonly used low molecular weight emulsifiers, i.e., lecithin, citrem and mono- and diglycerides, and casein or whey protein have been examined (Drapala et al., 2017; Liu et al., 2020; Zou & Akoh, 2013). However, the impact of the complex interplay of formulation components and in particular the molecular structure of the emulsifiers on interfacial characteristics and stability of spray-dried emulsions including its dependence on temperature stress has not been investigated.

This study focuses on the impact of interactions of emulsifying constituents with each other and medium chain triglyceride oil on the interfacial characteristics in emulsions for spray drying, their behaviour during processing and powder properties. As common examples of emulsifying constituents lecithin, citrem and mono-/diglycerides and whey protein isolate are used. The interfacial network and intermolecular interactions are evaluated using interfacial shear rheology.

It is hypothesized that all emulsifying constituent combinations result in an interfacial film with lower viscoelasticity in comparison with the whey protein stabilized film due to non-attractive interactions between low molecular weight emulsifiers (LMWE) and whey protein. This weakened interfacial network will facilitate oil droplet breakup during processing steps whereby a highly interfacial active LMWE will be able to stabilize these oil droplets and maintain the encapsulation efficiency in the powder.

Powders were subject to temperature stress ranging from -18 °C and 60 °C during 24 weeks of storage. During storage, temperature stress induces phase changes and thus affects powder properties depending on the interfacial film characteristics. High temperature stress at 60 °C will induce protein fat complexes, Maillard reaction and glass transition which will result in a change of powder characteristics. During low temperature stress at -18 °C, full crystallization occurs and LMWE with saturated fatty acid chains will promote release of encapsulated oil.

Powders were characterised through analyses of the oil droplet size distribution of the reconstituted emulsion and encapsulation efficiency. These powder characteristics can be correlated to the interfacial properties. Furthermore, crystallinity was analyzed via x-ray powder diffraction (XRPD), morphology by SEM and colour development over storage time. The investigation of microstructure and colour of the powder helps to identify oil at the particle surface and Maillard reactions of the powder matrix.

III.2 Materials and methods

III.2.1 Materials

For the preparation of spray-dried emulsions, whey protein isolate (WPI) (Lacprodan DI-9224, Arla Foods Ingredients Group P/S, Viby, Denmark), maltodextrin with a dextrose equivalent of 14 (C* Dry ™ MD 01910, Cargill Deutschland GmbH, Krefeld, Germany) and medium-chain-triglyceride oil (MCT-oil, WITARIX® MCT 60/40, IOI Oleo GmbH, Hamburg, Deutschland) were used. The WPI consisted out of 89.5% protein,

< 0.05% lactose, 0.1% fat, 5% moisture and < 4% ash. The fatty acid composition of the MCT-oil was composed of C 10:0 und C 8:0 fatty acids.

For the interfacial rheological analysis, β-LG was isolated from whey protein isolate (Bipro, Agropur Dairy Cooperative Inc., Minnesota, USA). The method for purification is described elsewhere (Keppler et al., 2014; Schestkowa et al., 2020). The resulting protein had a dry matter content of 90.7 ± 1.0% and a protein content of 90.1 ± 1.2% while the protein content is composed of 98.1% isolated β-LG, 0.4% α-lactalbumin and 1.5% denaturated β-LG (analyzed according to Keppler, Sönnichsen, Lorenzen, & Schwarz, 2014). Medium-chain-triglyceride oil (MCT-oil) WITARIX® MCT 60/40 was kindly provided from IOI Oleo GmbH (Hamburg, Germany) and was purified via magnesium silicate adsorption (Florisil®, Carl Roth GmbH, Karlsruhe, Germany) to remove interfacial active substances. Maltodextrin with a dextrose equivalent of 14 (C*Dry ™ MD 01910) was purchased from Cargill Deutschland GmbH (Krefeld, Germany). The maltodextrin had a protein content of 0.1-0.15% (measured with DUMATHERM, C. Gerhardt GmbH & Co. KG, Königswinter, Germany).

For both, spray-dried emulsions, and interfacial rheological analysis, three LMWE were used: citrem (GRINDSTED® Citrem N 12 Veg MB, Danisco, DuPont de Nemours Inc. Nutrition Biosciences ApS, Copenhagen, Denmark), mono- and diglyceride (Lamemul K 2000K, BASF SE, Illertissen, Germany) and lecithin (Metarin PB IP, Cargill Deutschland GmbH, Krefeld, Germany). More specifically, Citrem was a partially neutralized citric acid ester of mono- and diglyceride with almost fully hydrogenated fatty acids from palm-based oil. The mono- and diglyceride comprised 96% monoglycerides of fully hydrogenated fatty acids, also derived from palm oil. Therefore, both LMWE mainly consisted of C14:0, C16:0 and C18:0 with increasing concentration as known from the literature. The lecithin was derived from soy origin and thus is composed of unsaturated fatty acids with chain length and saturation of C18:1, C18:2, C18:3 and small portions of C16:0 according to the literature. The head groups consist of 2-9% phosphatic acid, 18-27% phosphatidylcholine, 10-16% phosphatidyl-ethanolamine, 14-19% phosphatidylinositol, 3% phosphatidylserine, 14-19% other phospholipids and 10-15% phytoglycolipids.

III.2.2 Preparation and spray drying of emulsions

Emulsions were prepared as described in Taboada et al. (2020). Briefly, the emulsions consist of 15 d.m.% (dry matter) MCT-oil with the ratio MCT oil to WPI and LME

1:0.1:0.01 and 24.8 d.m.% (dry matter) maltodextrin. The LMWE were solubilized in oil at 60 °C. An aqueous solution of WPI and the oil containing the LMWE were emulsified for 2 min in a colloid mill (IKA magic LAB®, IKA®-Werke GmbH & Co. KG, Staufen, Germany) operated at a gap width of 0.16 mm and a circumferential speed of 26 m s^{-1}. The maltodextrin was added after the homogenization process. Before spray drying, the emulsions were stored overnight to allow the interfacial film to stabilize.

Powders were produced from these emulsions with a spray dryer (Werco SD-20, FA. Hans G. Werner Industrietechnik GmbH, Reutlingen, Germany) and a pressure swirl atomiser of the type SKHN-MFP SprayDry® (core size 16, orifice diameter 0.34 mm, Spraying Systems Deutschland GmbH, Hamburg, Germany) at an inlet temperature of 195 °C and an outlet temperature of 75 °C. The drying air volume flow rate was 580 kg h^{-1}. The atomization pressure was set at 100 bar for a corresponding volume flow rate of 28.8 L h^{-1}. As all emulsions presented the same viscosity and dry matter content, the spray drying process was the same for all emulsions.

III.2.3 Storage of spray-dried emulsions

All powder samples were conditioned at a temperature of 30 °C and a relative humidity of 33% for 9 days in a climate chamber (KBF 115, Binder GmbH, Tuttlingen, Germany) and reached an aw-value of 0.35 ± 0.03 (measured with Labormaster aw neo, Novasina AG, Pfäffikon, Switzerland) and a dry matter of 95.1 ± 0.1% (Sartorius MA 30 Moisture analyser, Sartorius AG, Göttingen, Germany). After conditioning, aliquots of each powder were equally distributed and sealed in aluminium bags. Temperature stress for two weeks was conducted at -18 °C (- 20.5 ± 1.5 °C) or 60 °C (58.9 ± 1.1 °C). Afterwards, the samples were stored for 22 weeks at room temperature. A control was stored at room temperature (21.5 ± 1.2 °C). During storage time of 24 weeks, the temperature was controlled with data loggers (174 T Mini, Testo SE & Co. KGaA, Lenzkirch, Deutschland).

The extractable oil content, the oil droplet size distribution of the reconstituted powder, the crystalline structure via XRPD as well as colour and morphology of the powders were investigated at the start, day 0, and at the endpoint, 168 days, of storage.

III.2.4 Extractable oil of spray-dried emulsions

Extractable oil content was determined gravimetrically with petrol ether as solvent (Westergaard, 2004). Ten grams of powder were solubilized with 50 mL petrol ether in

a 100 mL Erlenmeyer flask and mixed for 15 min at 90 rpm on a shaking device. The dispersion was filtered, and 25 mL of the filtrate was transferred in dried and weighed round bottom flasks. The solvent was removed in a rotating evaporator at 65 °C and 700 mbar for 5 min. The evaporated round bottom flasks were weighed after heating for 90 min at 105 °C and cooling in a desiccator. The extractable oil content is provided as percentage of the emulsified oil. This content was measured for each sample at a storage time in duplicate and is shown as mean value with mean deviation.

III.2.5 Oil droplet size distribution of reconstituted powder

Oil droplet size distribution of the reconstituted powder was measured with laser diffraction (LA-950, Horiba Jobin Yvon GmbH, Bensheim, Germany). 2 g of powder were reconstituted in 20 g distilled water. This emulsion was stirred for 1 h at 250 rpm with a magnetic stirrer and measured six times at least. The measurement was performed at refractive index of material and dispersion material at 1.46 and 1.33, respectively. Results are reported as cumulative sum distribution curves which are volume based. The d_{50} and d_{90} of feed and reconstituted spray dried emulsion are shown as well. The coefficient of variation was estimated of four powders which were measured individually.

III.2.6 X-ray diffraction of spray-dried emulsions

The X-ray diffractor (XRPD) patterns were recorded with an X'PertPro (Malvern Panalytical GmbH, Kassel, Germany) with a reflection-θ-θ geometry at the chair of solid-state chemistry of Prof. Dr. Lerch at the Technische Universität Berlin. The method was used to verify the amorphous character of the powder after production and to identify a possible crystallization over time. The X-ray diffractometer was operated with samples on silicon wafer, at room temperature with 40 kV and 40 mA, at diffraction angles (2θ) from 10 to 80° with a step size of 0.013° with 30 s per step. The XRPD patterns were determined for each sample in single measurements.

III.2.7 Colour of spray-dried emulsions

The colour of the spray-dried emulsions was analyzed with Chromameter CR 300 (Minolta, Japan) using a CIELAB system (four measurements per sample at a storage time). Within the CIELAB colour space (L*, a*, b*), L* specifies the extent of lightness, a* indicates green-red and b* blue-yellow. The coefficient of variation was estimated for L*, a*, b* values of four powders which were measured individually.

III.2.8 Morphology of spray-dried emulsions

Morphology of the spray-dried emulsions was studied by a scanning electron microscope (S-2700, Hitachi, Tokyo, Japan) at the Centre for Electron Microscopy at the Technische Universität Berlin (ZELMI). For this purpose, the powders were gold sputtered with a coater SCD 030 (Balzers, Wiesbaden-Nordenstadt, Germany). Images were taken at 50 x, 300 x, 1000 x and 3000 x magnification for each formulation at every storage time.

III.2.9 Interfacial shear rheology

Interfacial shear rheology was performed with a Physica MCR301 and MCR102 rheometer (Anton Paar Germany GmbH, Ostfildern, Germany) equipped with an interfacial biconus (Bicone, Bi-C68-5, Anton Paar Germany GmbH, Ostfildern, Germany) at 20 °C. This method was used to determine the interfacial network and intermolecular interactions in the interfacial film (Krägel et al., 2008). Here, the major component of whey protein -β-lactoglobulin- was used as model protein. The isolated protein was chosen to ensure that effects in film behaviour can be attributed to interaction between the protein and the LMWE. The protein was applied at its critical interfacial concentration to ensure a monolayer of protein at the interface. The LMWE are used below their critical micelle concentration. Since LMWE at higher concentrations tend to displace proteins from the interface (Bos & van Vliet, 2001; Wilde et al., 2004), it is assured that both emulsifying constituents can coexist at the interface.

The protein/maltodextrin solutions were prepared at pH 7. The protein was dissolved and stirred in distilled water for approximately 2 h and reached a pH around 7. Maltodextrin was solubilized in distilled water with a stirring device (RCT Basic, IKA-Werke GmbH & Co. KG) for approximately 2 h. The pH was adjusted to 7 with 1 $_{M}$ NaOH. Protein and maltodextrin solutions were combined to obtain concentrations of 0.1% protein and 34.9 d.m.% maltodextrin. The solutions were stirred for further 3 h and were stored at 5 °C for about 14 hours overnight. Afterwards, all solutions were stirred to adjust temperature, pH and to obtain a homogenous solution before measurement. The LMWE were solubilized in purified MCT-oil to obtain a concentration of 0.005%.

The protein/maltodextrin solutions were carefully poured with the help of a glass rod into the interfacial shear glass cylinder. Bubbles were gently and immediately removed with Pasteur pipettes. The biconus was positioned directly at the interface and covered with a mixture of MCT-oil and low molecular weight emulsifier. The interfacial film development was monitored for 23 h at 1 Hz and 0.1% amplitude. The results are shown as development of the complex modulus (G*) over time. The samples were measured once. A coefficient of variation was estimated from 12 individual measurements.

III.3 Results

III.3.1 Oil droplet size distribution and extractable oil content of spray-dried emulsions

Prior to spray-drying the d_{50} and d_{90} of the oil droplet size in the liquid feed emulsion ranged from 2.50 ± 0.08 µm to 3.60 ± 0.17 µm and from 3.94 ± 0.18 µm to 5.50 ± 0.37 µm for WPI-lecithin, WPI-mono-and diglyceride, WPI-citrem and WPI alone, respectively (**Table III-1**, page 29). Oil droplet size decreased during the spray-drying process for all emulsifying constituent combinations and thus in the powder (**Table III-1**). WPI-lecithin based powder showed a distribution with smallest oil droplets followed by whey protein isolate, and by samples with addition of WPI and mono-and diglyceride or citrem (**Table III-1**). During storage, there was an increase in oil droplet size in the WPI-stabilized emulsion independent from the temperature stress (**Figure III-1**, page 28). In contrast WPI-lecithin stabilized emulsions did not show a change in oil droplet size. Samples stabilized with either WPI-citrem or WPI-mono- and diglyceride showed a slight increase in oil droplet size when stored at -18 °C (**Figure III-1**).

Presence of low molecular weight emulsifier also affected the content of extractable oil. In the presence of whey protein and lecithin the extractable oil content amounted to 6.2 ± 0.2% in comparison to the whey protein stabilized spray-dried emulsion with 7.4 ± 0.1%. In contrast, an increased extractable oil content of 9.5 ± 0.0% and 10.0 ± 0.0% was observed in spray-dried emulsions stabilized with WPI-mono- and diglyceride or WPI-citrem, respectively (**Figure III-2**, page 29). These differences in the extractable oil content between powders with different emulsifying constituent combinations remained in a similar order and range during storage. Generally, the extractable oil content remained similar or decreased over time. The only exceptions

were powder samples stabilized with WPI-citrem and WPI-mono- and diglyceride based powders stored at -18 °C. In these samples extractable oil increased up to 10.89 ± 0.38% and 10.26 ± 0.18%, respectively. In contrast, lowest extractable oil content induced by temperature stress was observed after 168 days of storage in samples with an initial temperature stress of 60 °C.

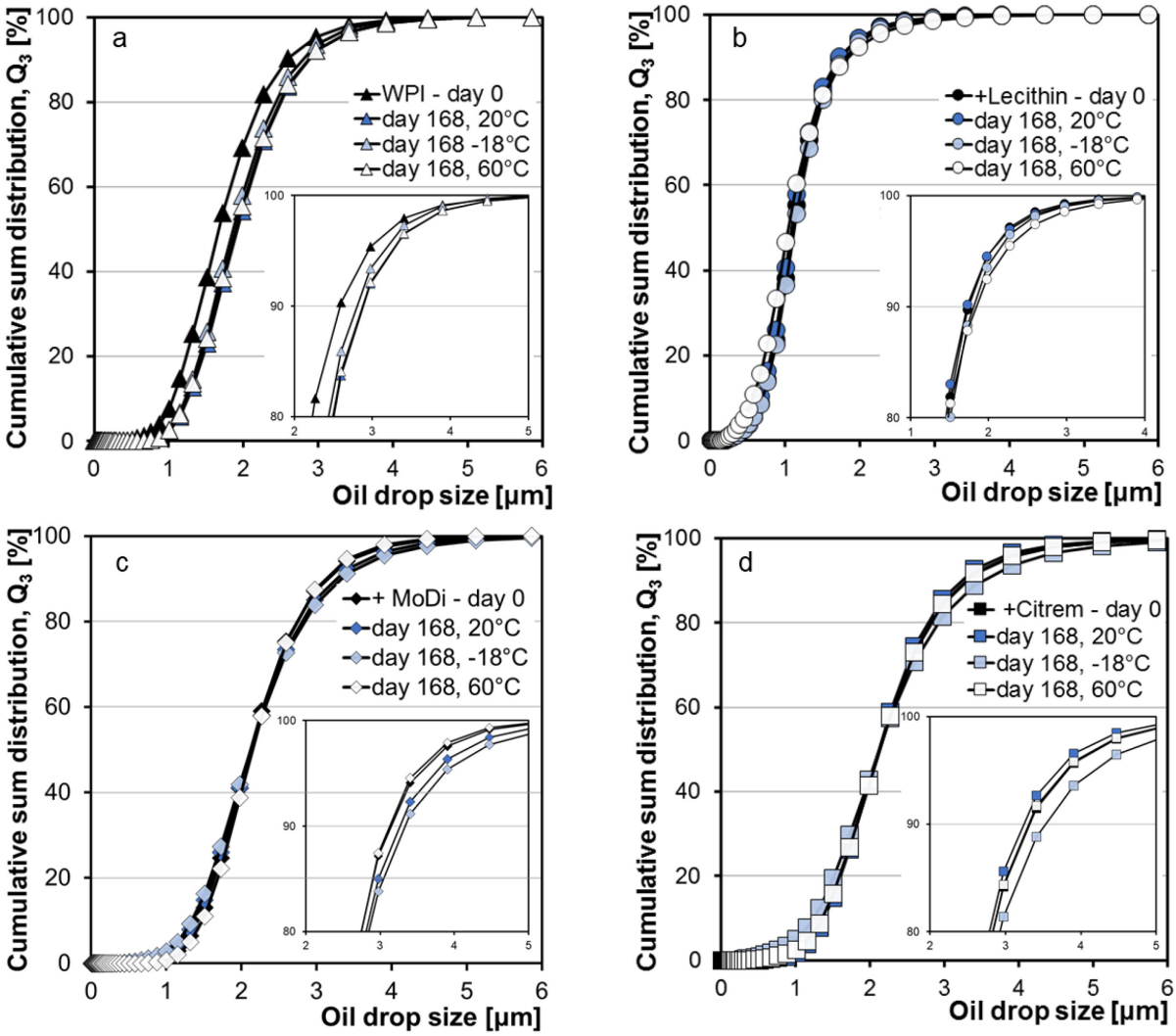

Figure III-1: Cumulative sum distribution of oil drop size of spray-dried emulsions which were stabilized by (a) whey protein isolate (WPI) with addition of (b) lecithin, (c) mono- and diglyceride (MoDi) and (d) citrem. The powders were analyzed at day 0 and after storage of 168 days (at 20 °C, -18 °C or 60 °C).

Table III-1: Oil droplet size of feed and reconstituted spray dried emulsions which were stabilized by whey protein isolate (WPI) with addition of lecithin, mono- and diglyceride (MoDi) and citrem[a].

Sample	Feed emulsion		Reconstituted spray dried emulsion	
	d_{50} [µm]	d_{90} [µm]	d_{50} [µm]	d_{90} [µm]
WPI	3.60 ± 0.17	5.50 ± 0.37	1.75 ± 0.08	2.64 ± 0.07
+ Lecithin	2.50 ± 0.08	3.94 ± 0.18	1.05 ± 0.02	1.80 ± 0.08
+ MoDi	3.30 ± 0.10	5.20 ± 1.04	2.10 ± 0.00	3.17 ± 0.06
+ Citrem	3.41 ± 0.47	4.94 ± 0.19	2.08 ± 0.01	3.15 ± 0.02

[a] Values are presented as mean ± standard deviation of the method.

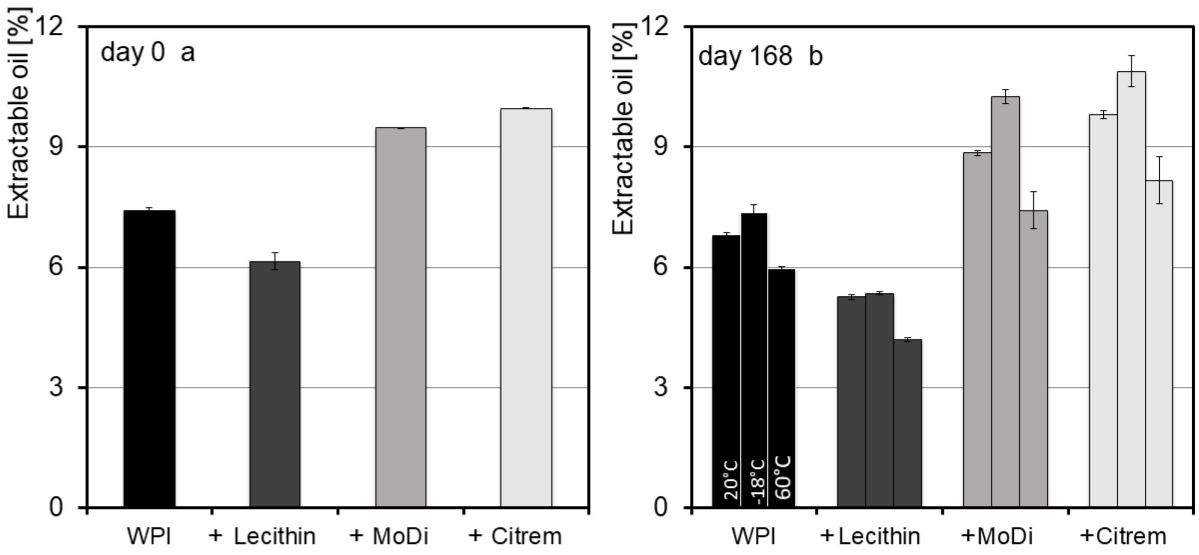

Figure III-2: Extractable oil of spray-dried emulsions which were stabilized by whey protein isolate (WPI) with addition of lecithin, mono- and diglyceride (MoDi) and citrem. The powders were analyzed at (a) day 0 or (b) after storage of 168 days (at 20 °C, -18 °C or 60 °C).

III.3.2 Morphology, crystallinity, and colour of spray-dried emulsions

SEM was used to determine the morphology of spray-dried particles with identification of surface oil. All spray-dried powders showed spherical particles with smooth to wrinkled surface. SEM revealed no visible difference in particle structure depending on combination of emulsifying constituents or temperature stress. Powders stored at - 18 °C are shown in **Figure III-3** at page 30. Some of the particles allow an insight in particle microstructure, which shows a porous appearance. Particle surface partly

shows regions with spreads indicated with circles in **Figure III-3**. Immediately after spray-drying and after 168 days of storage no crystalline material could be detected by X-ray diffraction. Diffraction pattern showed no distinct peaks, throughout the whole detection range (**Figure III-4**, page 31).

The colour of the powder was recorded via CIELAB. All samples show no difference in lightness (L*) which ranged from 92.9 ± 4.7 to 94.5 ± 4.7 at day 0. At day 0, the a* values range from -1.1 ± 0.1 to -1.3 ± 0.1 (**Table III-2**, page 32). The b* coordinate shows values from 0.6 ± 0.0 to 1.4 ± 0.1 (**Table III-2**). Over storage time, the L* value did not change. The a* value increased only for samples exposed to temperature stress at 60 °C from -1.5 ± 0.1 to -1.7 ± 0.1 for WPI, WPI-mono-and diglyceride, WPI-lecithin and WPI-citrem (**Table III-2**). The b* value slightly increased for all stored samples whereby the highest increase was shown for samples with temperature stress at 60 °C. For these samples, the b* value increased in a range from 6.4 ± 0.3 to 8.2 ± 0.4 for WPI-citrem, WPI-mono- and diglyceride, WPI-lecithin and WPI (**Table III-2**).

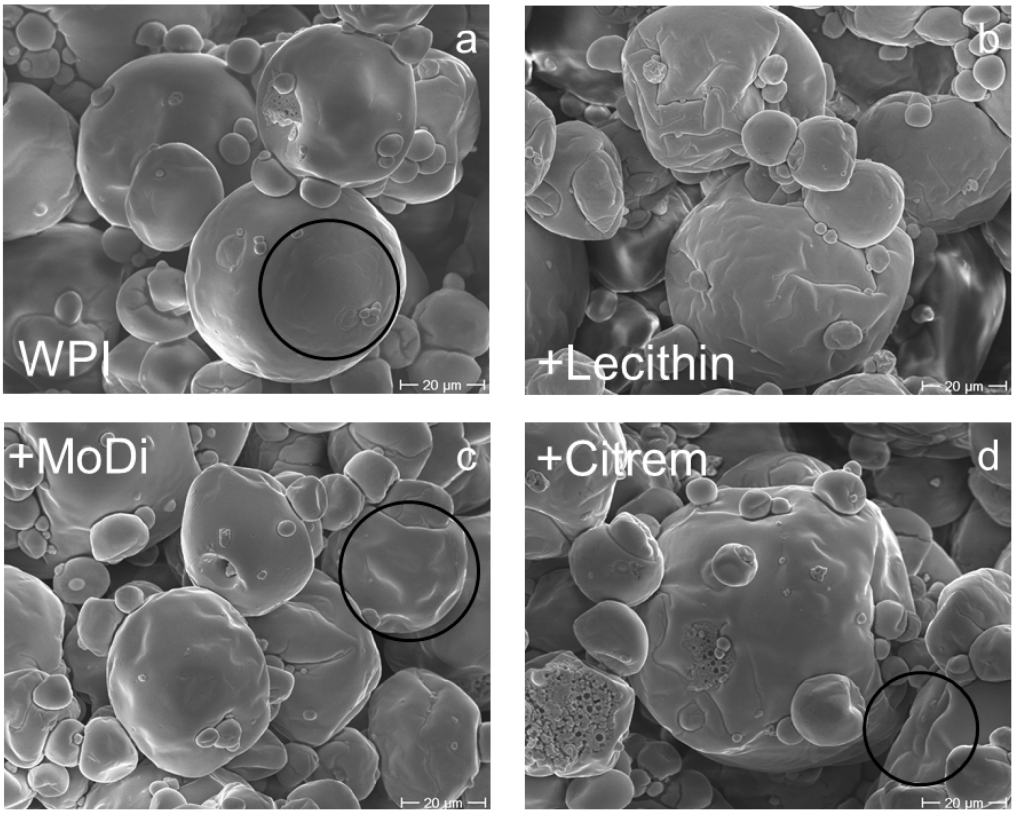

Figure III-3: Scanning electron microscopy images of spray-dried emulsions at 1000x magnification which were stored at -18 °C and were stabilized by (a) whey protein isolate (WPI) with addition of (b) lecithin, (c) mono- and diglyceride and (d) citrem. Black circles indicate regions with predominantly free fat at surface.

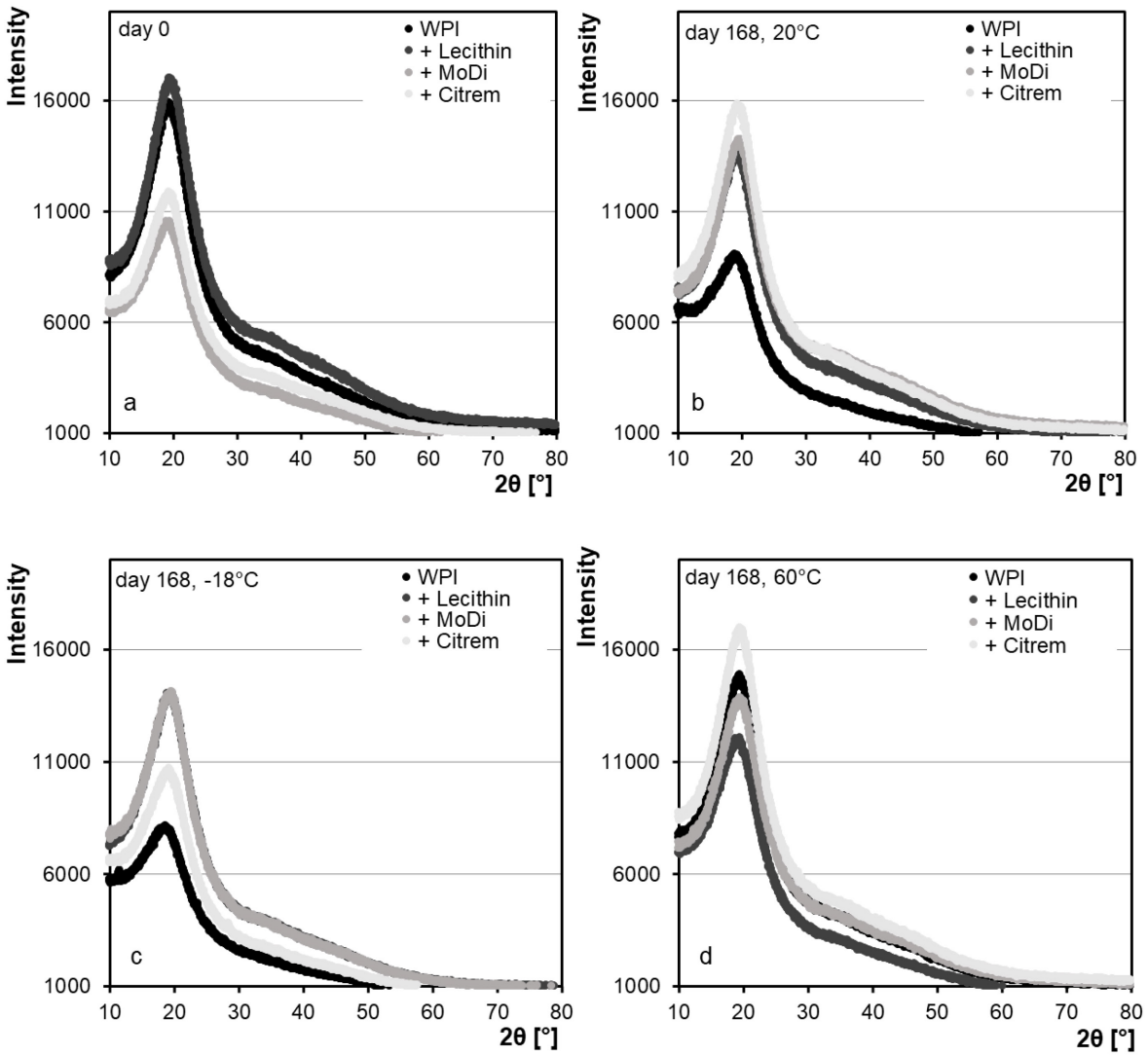

Figure III-4: X-ray diffraction (XRPD) patterns of spray-dried emulsions which were stabilized by whey protein isolate (WPI) with addition of lecithin, mono- and diglyceride and citrem. The powders were analyzed at (a) day 0 or after storage for 168 days at (b) 20 °C, (c) -18 °C or (d) 60 °C.

Table III-2: Colour (CIELAB a* and b*) of freshly prepared (day 0) and stored (day 168, -18 °C, 20 °C or 60 °C) spray-dried emulsions stabilized with whey protein isolate (WPI) and under addition of lecithin, mono- and diglyceride (MoDi) and citrem [a].

Sample	**day 0**		**day 168**					
			-18°C		20°C		60°C	
	a*	b*	a*	b*	a*	b*	a*	b*
WPI	-1.18 ± 0.06	0.76 ± 0.04	-1.28 ± 0.06	1.36 ± 0.07	-1.26 ± 0.06	1.08 ± 0.05	-1.54 ± 0.08	8.16 ± 0.41
+ Lecithin	-1.30 ± 0.06	1.38 ± 0.07	-1.42 ± 0.07	2.07 ± 0.10	-1.44 ± 0.07	2.33 ± 0.12	-1.68 ± 0.08	7.58 ± 0.38
+ MoDi	-1.16 ± 0.06	0.85 ± 0.04	-1.16 ± 0.06	0.90 ± 0.05	-1.17 ± 0.06	1.04 ± 0.05	-1.65 ± 0.08	7.38 ± 0.37
+ Citrem	-1.12 ± 0.06	0.58 ± 0.03	-1.21 ± 0.06	1.07 ± 0.05	-1.25 ± 0.06	1.18 ± 0.06	-1.72 ± 0.09	6.37 ± 0.32

[a] Values are presented as mean ± standard deviation of the method.

III.3.3 Interfacial shear rheology

Interfacial shear rheology is applied to analyze the viscoelastic interfacial network and intermolecular interactions at the oil-water interface. **Figure III-5** shows the development of the complex shear modulus G* over time for all combinations of emulsifying constituents at the oil-water interface with presence of maltodextrin. The β-lactoglobulin stabilized interface reaches a G* of approximately 30 mN m^{-1} (**Figure III-5**). The β-lactoglobulin-lecithin stabilized system showed the lowest G* of 6.4 mN m^{-1} with no change over time. The β-lactoglobulin-citrem and β-lactoglobulin-mono- and diglyceride stabilized interface both showed an initial increase in G* up to 20 and 16 mN m^{-1}, respectively with a slow decrease over time.

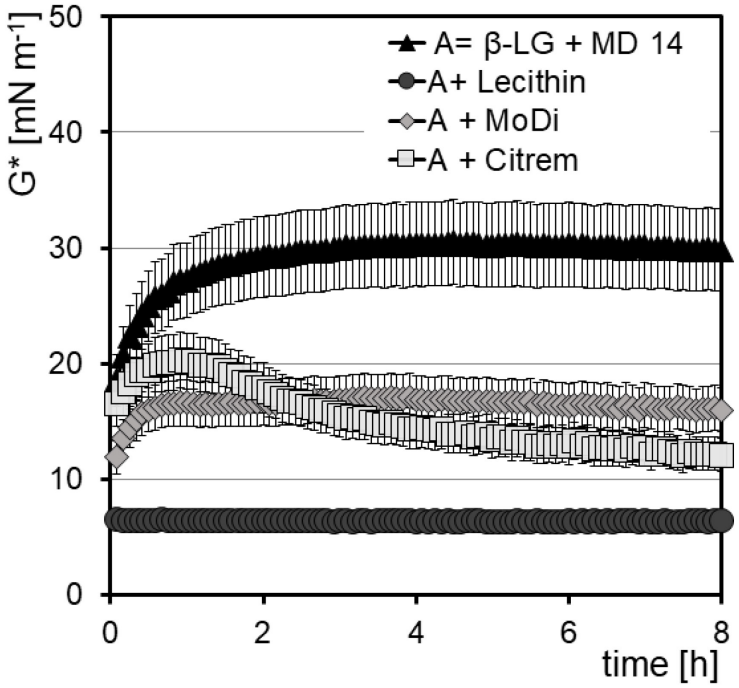

Figure III-5: Complex modulus (G*) of 0.1% β-LG film with addition of maltodextrin DE 14 (MD 14) in the aqueous phase and with addition of 0.005% lecithin, mono- and diglyceride and citrem in the oil phase, measured at oil/ water-interface, 1 Hz and 0.001 amplitude. Error bars display the coefficient of variation of the method.

III.4 Discussion

Selection of the emulsifying constituent already affected the oil droplet size distribution in the feed emulsion. Combination of WPI with LMWE decreased the d_{50} and d_{90} of the oil droplet size distribution compared to WPI alone (**Table III-1**, page 29). During emulsification, the combinations of emulsifying constituents reduce the oil droplet size of the whey protein stabilized emulsion in dependence on their interfacial activity. It belongs to the well-established knowledge that LMWE have in general a higher interfacial activity than proteins (Murray & Dickinson, 1996) and thus more efficiently stabilize the newly created droplets during homogenisation. Differences in the interfacial tension of the emulsifier constituent combinations of the present study have already been shown (Taboada et al., 2020). The interfacial tension was lowest for WPI-lecithin followed by WPI-mono- and diglyceride and WPI-citrem (Taboada et al., 2020).

All spray dried emulsions were amorphous (**Figure III-4**, page 31) with spherical particles and smooth to wrinkled surface (**Figure III-3**, page 30) comparable to spray-dried emulsions which have been shown previously (Masum et al., 2019). However, the spray dried emulsions differed in oil droplet size distribution and extractable oil content. These differences in the physicochemical characteristics result from differences in interfacial properties, phase transition phenomena within the oil phase and the matrix material upon temperature stress or molecular interactions occurring at elevated temperature.

In general, in all samples the oil droplet size decreased during spray-drying and particle formation (**Table III-1**, page 29), which can be attributed to oil droplet break up during atomization (Taboada et al., 2020). It belongs to the well-established knowledge that in this context a viscoelastic interfacial film preserves the stability of spray dried emulsions (Vega & Roos, 2006). Emulsions are typically stored prior to spray drying and this was also the case in the present study. Monitoring the rheological behaviour of the interfacial film over a prolonged period of time is therefore a suitable technique to reveal differences when using combinations of emulsifying constituents. In the present study, the whey protein stabilized interface showed the highest G* indicating that it has the highest viscoelasticity among all samples. It results from strong intermolecular interactions (Murray & Dickinson, 1996; Wilde et al., 2004). Furthermore it is supported by protein enrichment at the interface (Rodríguez Patino & Pilosof, 2011)

due to an excluded volume effect as it has been described for β-lactoglobulin and maltodextrin (Heiden-Hecht, Ulbrich, et al., 2021).

Addition of LMWE to a protein stabilized system exhibited a reduction in the viscoelasticity of the interfacial protein film in the present study. The reduction in viscoelasticity can be mainly explained by partial protein displacement (Bos & van Vliet, 2001; Wilde et al., 2004), electrostatic repulsion (Lam & Nickerson, 2013) and other non-attractive interactions of proteins and LMWE (Crespo-Villanueva et al., 2018; Dan et al., 2013; Kotsmar et al., 2009). This reduction in G* can be attributed to a weak interfacial film which facilitates breakup during atomization in comparison to a viscoelastic protein film.

In addition, if the newly created interface is not stabilized by the emulsifying constituent, oil droplet coalescence may occur and lead to a shift in the oil droplet size distribution towards an increased oil droplet size. Furthermore, coalescence may occur during water evaporation and particle formation, when oil droplets approach each other due to a reduction of the volume by evaporation. In this scenario a highly elastic behaviour of the interfacial film offers protection against unintended changes in oil droplet size. It becomes obvious that the properties of the interfacial film of the emulsifying constituents play a key role during the atomization induced break up and potential coalescence. In the present study, the highly interfacial active low molecular weight emulsifier lecithin prevented coalescence and maintained the decrease in oil droplet size. In comparison, in the presence of whey proteins or combinations of WPI and LMWE with a lower interfacial activity like mono- and diglycerides or citrem, oil droplet coalescence occurred to a varying degree and led to a larger oil droplet size as it was earlier described elsewhere (Taboada et al., 2020). A large oil droplet size went hand in hand with a high extractable oil content and vice versa as it becomes obvious when comparing the results in **Table III-1** and **Figure III-2**a at page 29. WPI-lecithin showed the smallest oil droplet size and the lowest extractable oil content followed by WPI, WPI-citrem and WPI-mono- and diglyceride (**Table III-1** and **Figure III-2**, page 29). We assume that after the oil droplet break up during atomization and subsequent coalescence of oil droplets another factor might play a role. Since the time scale from atomization to powder particle formation of spray dried emulsions takes just milliseconds (Taboada et al., 2019; Vega & Roos, 2006), non-stabilized regions of oil droplets may be especially present for WPI, WPI-citrem and WPI-mono- and

diglyceride. These non-stabilized regions tend to be not well encapsulated and thus merge with the matrix material. The non-encapsulated oil migrates to the surface or stays in the matrix and can be determined via solvent extraction (Vignolles et al., 2007). A powder with a high extractable oil content is more prone to aggregation and shows a reduced solubility. Therefore, from a practical point of view a low content of extractable oil is a key factor in quality evaluation of spray-dried emulsions.

During storage, changes in oil droplet size distribution and extractable oil content depended on emulsifying constituent combination and temperature stress (**Figure III-1**, page 28 and **Figure III-2**, page 29). For both, a reduction or an increase of extractable oil content, mechanistic explanations are available. In the present study in WPI-citrem and WPI-mono- and diglyceride stabilized systems exposed to temperature stress at – 18 °C, the extractable oil content and the oil droplet size increased (**Figure III-1**, page 28 and **Figure III-2**, page 29). In general, crystallization in emulsions requires supercooling, i.e., crystallization temperature is well below the crystallization temperature of the bulk material. Homogeneous nucleation within the oil phase is less likely than heterogeneous nucleation (Garti & Sato, 2001). It is well accepted, that due to the low volume of the oil droplets in an emulsion, volume heterogeneous nucleation due to impurities in the oil is also rare. The major driver for nucleation thus is the so-called surface heterogenous nucleation, where the emulsifier acts as a template for crystallization (McClements, 2012; Ribeiro et al., 2015). When triacylglycerols crystalize, they usually form α-polymorph, since it is the polymorph with the lowest activation energy, but not necessarily the lowest free energy (McClements, 2012; Ribeiro et al., 2015). As a consequence, polymorphic transitions occur from the α-polymorph through the β'-polymorph to the most stable β-polymorph (McClements, 2012; Ribeiro et al., 2015). Again, polymorphic transitions in an emulsion are much faster than in the bulk material due to a smaller crystal size, and the oil-water interface represents a physical barrier hindering growth (McClements, 2012). In a liquid emulsion polymorphic transition leads to a change in crystal shape from a spherical to a more ellipsoid shape (Awad et al., 2008; McClements, 2012) and crystals may pierce the interface and induce oil droplet aggregation and coalescence (Fredrick et al., 2013; Goibier et al., 2017). Although this deformation may not occur in spray-dried emulsions and mobility of the oil droplets is prevented by their immobilisation in the amorphous matrix, piercing with release of oil may still occur during storage and result in release of encapsulated fat (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003).

Recrystallization upon storage at -18 °C and its impact on extractable oil differed depending on the emulsifying constituent combination in the present study. There was no change in extractable oil content in WPI-based spray dried emulsions stored at - 18°C. This is in accordance with the literature stating that proteins are not expected to catalyse triacylglycerol nucleation through any form of molecular similarity or incorporation into a compound crystal (Garti & Yano, 2001). The same holds true for the WPI-lecithin stabilized spray-dried emulsion in the present study. With the majority of the fatty acids being long chained and unsaturated, the crystallization temperature is not in a suitable range for serving as a template for medium chain triglycerides. Frederick et al. (2013) emphasise that chain crystallization of the low molecular weight emulsifier is a prerequisite in heterogeneous nucleation and thus needs to precede triacylglycerol crystallization (Fredrick et al., 2013). It is in line with the observation of Garti & Yano that, e.g., a template would occur for an intermediate insoluble LMWE with a longer fatty chain length, a higher crystallization temperature than the surrounding and most likely unsaturated oil phase (Garti & Yano, 2001). Thus, in the present study templating and fast crystallization with an increase of extractable oil content was observed in WPI-citrem and WPI-mono-diglyceride systems, which contained saturated fatty acids with a chain length of 14 to 18 C-atoms.

In contrast, in all samples exposed to temperature stress at 60 °C, the extractable oil content decreased. This decrease in extractable oil may result through formation of protein-fat complexes (Vignolles et al., 2007). The polypeptide chain can interact with the fat in dependence on structural aspects of the protein (Brinkmann et al., 2013). The authors highlight that protein-fat complexes are very likely for an oil phase and whey proteins at 60 °C (Brinkmann et al., 2013; Lišková et al., 2011). This leads to the conclusion that interactions are hydrophobic in nature and steric effects must also contribute to get a markable effect on extractable oil content. Furthermore, this decrease can be attributed to phase transition phenomena of the matrix material (Roos, 2002; Roos & Karel, 1991; Zafar et al., 2017). For a matrix material of maltodextrin with DE 14 and an a_w-value of 0.35, the glass transition temperature is around 60 °C (Roos & Drusch, 2016). Since the DE is a degree of starch degradation without specific molecular weight profile for the maltodextrins, the differences in molecular weight in the matrix material can induce local phase transition (Hughes et al., 2018). A reduction in extractable oil content due to phase transition of the matrix material could be attributed to the mechanisms of caking. These mechanisms can be,

e.g., bridging between particles (Zafar et al., 2017) which can difficult the extractability of the oil in a short and controlled solvent residence time applied in our study.

Beside the reduction of the extractable fat content, at 60 °C Maillard reaction seems to be very likely for a powder containing whey protein isolate and reducing sugars stored at 60 °C (Schmitz et al., 2011). The Maillard reaction could be attributed to the increase in a* and b* values for high temperature stressed samples (**Table III-2**, page 32). In earlier studies, a similar b* value of 7 to 8 was associated with Maillard reaction in spray-dried emulsions with lactose-maltodextrin mixtures (Masum et al., 2019).

III.5 Conclusion

Interfacial properties of emulsifying constituent combinations and interactions with the oil phase influence the physical properties of spray dried emulsions and changes during storage. The interfacial properties depend on interactions of emulsifying constituents in the interfacial film and interactions with the oil phase. Therefore, at the interface electrostatic, hydrophobic and hydrophilic interactions play a key role as well as phase transition in the oil phase. However, to improve our understanding a systematic approach is required with targeted combination of the fatty acid composition of the oil phase and LMWE with defined fatty acid composition and head group. In this approach analytical techniques for monitoring of crystallization phenomena in model systems and in situ are required. X-ray patterns have been used to monitor the overall crystallinity in the present study but are not suitable to specifically monitor the interface and emulsion droplets. According to a recent XRPD review, the identification of crystals can be difficult if their size is too small or if they are mixed with other ingredients in a low amount (Holder & Schaak, 2019). Suitable techniques to define crystal structure, form, size and position in the emulsion system comprise SANS, SAXS or NMR (Bernewitz et al., 2011; Yesiltas et al., 2019). The results will lead to an improved understanding of emulsion characteristics and behaviour and thus will help to enhance storage stability of spray dried emulsions and tailor formulations of spray-dried emulsions for specific areas of application.

IV. Manuscript 2

Spray drying of emulsions: Influence of the emulsifier system on changes in oil droplet size during the drying step

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Abstract

The goal of this study was to investigate the influence of the emulsifier system on the changes in oil droplet size occurring during the drying step of spray drying of emulsions. Atomization and spray drying experiments were performed with emulsions stabilized with whey protein isolate (WPI) alone or in combination with low molecular weight emulsifiers (lecithin, mono- and diglycerides (MoDi) and citrem). Oil droplet coalescence was observed for the systems WPI/Citrem and WPI/Modi, as the $d_{90,0}$ increased from 0.86 ± 0.16 µm and 1.67 ± 0.35 µm after atomization to 1.83 ± 0.24 and 1.90 ± 0.17 µm after drying, respectively. Oil droplets stabilized with WPI or WPI/Lecithin remained stable during drying. Measurements of dilatational rheology of the interfacial film showed that phase angle values increase in the order WPI/Lecithin < WPI < WPI/Citrem = WPI/MoDi. Therefore, in the studied system oil droplet coalescence during drying increases when the elastic behavior of the interfacial film decreases.

Practical applications

Spray drying of emulsions is a widely used process in the food industry for production of, for example, infant formula, dairy powders and encapsulated aroma and coloring compounds. The oil droplet size in the resulting powder determines sensory aspects and stability of the final product. This study deepens the understanding of the changes in oil droplet size occurring during spray drying as affected by the formulation components, allowing therefore a better control of the quality of spray dried food emulsions.

Keywords: spray drying, oil droplet size, coalescence, emulsions, emulsifier, dilatational rheology

IV.1 Introduction

A wide variety of food powder products with encapsulated oily components are produced via spray drying of oil-in-water emulsions. Examples include infant formula, instant dairy powders and products with encapsulated flavors and functional lipids (Gharsallaoui et al., 2007). Typical formulations include the oily phase to be encapsulated, a protein source (e. g., whey protein) acting as emulsifier (Prasad Reddy et al., 2019; Ramakrishnan et al., 2014), as well as a carbohydrate source (e.g., starch conversion product) acting as matrix material (Fang et al., 2019; Sanchez-Reinoso & Gutiérrez, 2017) after drying. Lipid-based, low molecular weight emulsifiers (LMWE) are also commonly added to formulations, as they are expected to improve the stability of emulsions during processing and storage by improving the characteristics of the adsorption layer around the oil droplets (Petrovic et al., 2010; Shujie Wang et al., 2017). LMWE commonly added to protein-based formulations include lecithins, mono- and diglycerides (MoDi), and esters of fatty acids (e.g., citrem) (Danviriyakul et al., 2002; Drapala et al., 2017).

In the first step of spray drying process, oil-in-water emulsions are atomized into fine droplets with a nozzle. In the subsequent drying step, the spray droplets are dried to powder upon contact with hot air (Barbosa-Cánovas et al., 2005; Hernandez Sanchez et al., 2015). The oil droplet size distribution (ODSD) in the powder influences the stability of the powder upon storage, as well as the functional properties of the reconstituted emulsion (Haas et al., 2019; McClements & Li, 2010) and is therefore an important quality parameter. In industrial processes, an emulsification step is applied prior to spray drying to adjust the ODSD to the desired product-specific value. However, previous studies have shown that changes in ODSD may take place during spray drying process (Gharsallaoui et al., 2010; Serfert et al., 2013; Taneja et al., 2013). The addition of LMWE to protein based emulsifiers can greatly influence the extent of these changes. For example, Drapala et al. (Drapala et al., 2017) observed a significant increase in the oil droplet size after spray drying of emulsions when combining whey protein hydrolysate (WPH) with a citrem or a lecithin, as compared to emulsions stabilized with WPH alone.

In the named studies, the changes in ODSD were investigated by comparing the ODSD before the complete spray drying process with the ODSD after the complete spray drying process and powder reconstitution. No study has been found in which the

phenomena occurring during each step of the spray drying process, namely the atomization of the liquid followed by the drying of the spray droplets were studied separately. In our preceding study, the changes in ODSD occurring during the atomization step in dependence of the emulsifier system were investigated (Taboada et al., 2020). We focused on the effects of adding lipid-based LMWE (lecithin, citrem, MoDi) to whey protein stabilized emulsions. The results showed that oil droplet breakup takes place during the atomization step, almost independently of the emulsifier system. Immediately after breakup in the nozzle, coalescence of the newly created oil droplets may take place. These phenomena are largely influenced by the emulsifier system (Taboada et al., 2020). In the preceding study, the changes in oil droplet size during the drying step remained unaccounted for. But, during the drying step of spray drying, the oil droplets are forced close to each other due to water evaporation and volume reduction. Therefore, it is likely that coalescence of the oil droplets is further promoted. Coalescence during the drying step would lead to further changes in the oil droplet size.

The changes in oil droplet size during drying are expected to be strongly influenced by the interfacial behavior of the emulsifier system and by the viscoelasticity of the interfacial film. Proteins and LMWE show differences in interfacial stabilization. Proteins may form a viscoelastic layer at the interface which operates as a physical barrier against coalescence (Wilde et al., 2004). Therefore, we expect that oil droplets stabilized with WPI remain better protected against coalescence during the drying step. When proteins are aggregated, they can also stabilize emulsions by forming pickering emulsions (Burgos-Diaz et al., 2020). In this study we focused on native proteins as raw material and therefore this mechanism is not further considered. LMWE have a higher interfacial activity than proteins, but do not form viscoelastic layers (Bos & van Vliet, 2001). In general, a mixed interfacial film of LMWE and protein tends to show a reduced viscoelasticity compared to protein films, which can be explained by protein displacement and loss in interfacial interactions (Murray & Dickinson, 1996). Thus, increased coalescence is expected during the drying step with combinations of WPI/LMWE. However, a combination of both emulsifier types may result in more complex interfacial mechanisms influenced by interfacial activity, electrostatic and hydrophobic effects (Dan et al., 2013; Kotsmar et al., 2009), which influence the interfacial tension and viscoelasticity of the interfacial film. Therefore, the effects on the interfacial tension and viscoelasticity are not straightforward. The effects of proteins

and LMWE on viscoelasticity of interfacial films can be estimated with dilatational rheology. In these measurements, the interfacial film is characterized by response of the interfacial area to expansion and compression (Lucassen-Reynders, 1993).

The goal of the present study was to investigate the influence of the emulsifier system (WPI vs. WPI/LMWE) on the changes in oil droplet size during the drying step of spray drying process. For this, atomization and spray drying experiments were performed in pilot scale with emulsions stabilized with WPI or combinations of WPI/LMWE. By comparing the ODSD after atomization and after spray drying, the changes in oil droplet size were quantified. Furthermore, the observed changes were explained via changes of interfacial tension and viscoelasticity of the interfacial film, characterized with pendant drop tensiometry and dilatational rheology.

IV.2 Materials and methods

IV.2.1 Model emulsions: Preparation and characterization

Oil-in-water emulsions were prepared for the investigations. Medium-chain triglycerides oil was used as dispersed phase (MCT oil, WITARIX® MCT 60/40, IOI Oleo GmbH, Hamburg, Germany). Whey protein isolate (WPI, Lacprodan DI-9224, Sønderhøj, Denmark) served as protein emulsifier. The WPI composition was as follows: 89.5% protein, < 0.05% lactose, 0.1% fat, 5% moisture and < 4% ash. A soybean lecithin (Metarin, Cargill, Hamburg, Germany), a citrem (GRINDSTED CITREM N12, DuPont Nutrition & Biosciences, Brabrand, Denmark) and mono- and diglycerides (Lamemul K 2000 K, BASF Personal Care and Nutrition GmbH, Monheim, Germany) were used as lipid-based LMWE. The citrem is a partially neutralized citric acid ester of mono-diglyceride with almost fully hydrogenated palm-based oil fatty acids. The mono- and diglyceride has fully hydrogenated fatty acids with head groups of 96% monoglyceride. The lecithin consists of a mixture of headgroups with decreasing percentage: phosphatidylcholine, phosphatidylinositol, phosphatic acid and phosphatidylserine. As matrix material maltodextrin (C*DryTM MD 01910, Cargill^{TM}, Haubordin, France) was chosen.

Emulsions were prepared following the procedure described in (Taboada et al., 2020). Briefly, emulsion premixes (50 wt.% oil) consisting of an aqueous WPI solution and MCT oil with LMWE (lecithin or citrem or MoDi) were prepared and homogenized in a colloid mill (IKA magic LAB®, IKA®-Werke GmbH & Co. KG, Staufen, Germany)

operated at a gap width of 0.16 mm and a circumferential speed of 26 m/s. The emulsion premixes were then mixed with the continuous phase, namely a solution of maltodextrin in water, to obtain the emulsions for atomization and spray drying experiments. This procedure was performed to produce a large volume of emulsion with the exact oil droplet size ensuring constant start conditions for all experiments. The oil content in the final emulsions was 15 wt.% and the ratio of MCT oil to WPI and LMWE was 1:0.1:0.01. These concentration ratios are in the range for spray drying applications of emulsions (Drapala et al., 2015). The concentration of maltodextrin in the final emulsion was 24.8 wt.%. The reported mass fractions refer to the total emulsion. As comparison, emulsions without added lipid-based LMWEs were also prepared.

The oil droplet size of the emulsions was measured via laser diffraction (HORIBA LA950, Retsch Technology GmbH, Haan, Germany). The data were analyzed by the Mie theory with a standard optical model for MCT oil in water. The $d_{90,0}$ (90 %-value of number based distribution) was chosen as characteristic value to analyze differences in oil droplet sizes. Viscosities of the emulsions were measured at 20 °C by rotational rheometry (Physica MCR 101, Anton Paar, Graz, Austria) using a double gap geometry (DG26.7). A logarithmic shear rate-controlled ramp was performed between 1 and 1000 s^{-1}. Emulsions were stored overnight (12 hr) before atomization or spray drying. Preliminary investigations showed that the oil droplet size remains constant for all emulsions during this time span.

IV.2.2 Atomization of emulsions

To determine the oil droplet size after atomization, experiments were performed in a pilot-scale spray test rig. A detailed description of the setup is provided elsewhere (Taboada et al., 2020). Briefly, a high pressure three-piston pump (Rannie LAB Typ 8.5, SPX FLOW Inc., Charlotte, USA) was used to supply the emulsions to a pressure swirl atomizer of the type SKHN-MFP SprayDry (core size 16, orifice diameter 0.34 mm, Spraying Systems Deutschland GmbH, Hamburg, Germany). Emulsions were tempered to 20 °C and atomized at a pressure of 100 bar and a corresponding volume flow rate of 28.8 L/h. During atomization, a sample of the spray was taken with a beaker 25 cm below the nozzle.

The spray test rig was also equipped with an in-line laser diffraction spectroscope (Spraytec, Malvern Instruments GmbH, Herrenberg, Germany) which allowed the

measurement of the spray droplet size distribution (SDSD) during atomization. Spray droplet sizes were measured 25 cm underneath the nozzle exit for 30 s. A time-average SDSD was calculated. SDSD are of great relevance for the drying behavior as they determine the area for heat and mass transfer during the drying process.

Atomization experiments were performed in duplicate trials with two separately prepared emulsions. Two samples were taken at each trial, resulting in six independent samples for analysis.

IV.2.3 Spray drying of emulsions

Spray drying experiments were performed in a pilot-scale spray dryer (Werco SD20, Hans G. Werner Industrietechnik GmbH, Germany) using the same atomization conditions as in the atomization experiments. The spray dryer was operated with an inlet and outlet temperature of 195 and 75 °C, respectively. The corresponding air volume flow was 580 kg/h. The resulting powders were collected and stored in air-tight containers until analysis. Spray drying experiments were performed in duplicate with two separately prepared emulsions. Comparison of ODSD in emulsions after atomization (from **chapter IV 2.2**) with ODSD after spray drying allows the quantification of the effect of the drying step on the oil droplet size.

IV.2.3.1 Powder analyses

To determine the oil droplet size after spray drying, powders were dispersed in water under gentle magnetic stirring (0.1 g/ml). The oil droplet size of the reconstituted emulsion was determined via laser diffraction as described in the previous section. Scanning electron microscopy (SEM, FEI Quanta 650 ESEM) was further used to study the powder microstructure.

Powders were also characterized by their particle size distribution (PSD), moisture content and water activity. PSD of the powders were measured by a laser diffraction spectroscope with powder dispersion unit (HORIBA LA950, Retsch Technology GmbH, Haan, Germany). In this device, the powder was dispersed in the measurement chamber with a gas flow at a pressure of 2.5 bar. Moisture content was analyzed by weight loss after oven drying at 105 °C to constant mass. Water activities were measured by a dedicated instrument (LabMaster-aW Neo, Novasina, Switzerland).

All measurements were performed in triplicate. The data was analyzed by 1-way-ANOVA with a significance level of $p < 0.05$ using the software OriginPro 2018

(OriginLab Corporation, Northampton, USA). Scheffè's test was used for mean comparison.

IV.2.4 Dilatational rheology

The interfacial behavior of the emulsifier system was characterized by determination of the interfacial tension and viscoelasticity of the interfacial film. Therefore, a pendant drop tensiometer (PAT1M, Sinterface Technologies e.K., Berlin, Germany) with a high-speed camera was used at 22 °C.

For these experiments, the major component of WPI - β-lactoglobulin - was utilized as model protein to ensure a high accuracy and precision of the of the results by reducing the noise in the measurements caused by the other numerous components in WPI. Typical values of β-lactoglobulin content in commercial WPI are between 45% and 69% (Foegeding et al., 2011). β-lactoglobulin (β-LG) was isolated from WPI (Bipro, Agropur Dairy Cooperative Inc., Minnesota, USA) with a purity of 98.11% (analyzed according to (Keppler et al., 2014). The protein was used at its critical interfacial concentration to provide a monolayer of protein at the interface (Tamm et al., 2012). LMWE and MCT-oil were utilized as described in **chapter IV 2.1**. The LMWE were used below their critical micelle concentration. Since LMWE are able to displace proteins from the interface (Wilde et al., 2004), it was assured that both emulsifiers share the same interface. The applied concentration ratio was the same as in the emulsions for spray drying experiments. The MCT-oil was purified via magnesium silicate adsorption (Florisil®, Carl Roth GmbH, Karlsruhe Germany) to remove interfacial active substances.

The protein solutions were prepared at pH 7. Therefore, the protein was dissolved and stirred in distilled water for approximately 2 hr. The pH was adjusted to 7 with 1 M NaOH. The LMWE were solubilized in purified MCT oil to obtain a concentration of 0.005 wt.%. During the measurement, a drop of protein solution with a volume of 30 mm^3 was formed in purified MCT oil with or without addition of LMWE. The drop was equilibrated for 14 hr and the interfacial tension was recorded. Afterwards, a frequency sweep (2.8% amplitude, 0.001 to 0.1 Hz) was performed. In this study, the results of dilatational rheology are expressed with the phase angle (ϕ) as important key parameter for elastic and viscous behavior. A phase angle of 0° indicates only elastic behavior of the interfacial film. If there is a phase angle of 90°, the interfacial

film reacts only viscous. A value between 0° and 90° shows a viscoelastic behavior of the film.

IV.3 Results and Discussion

IV.3.1 Feed emulsions characteristics and spray drying performance

The characteristic oil droplet sizes $d_{90,0}$ of the feed emulsions prior to atomization and spray drying are summarized in **Table IV-1**. Emulsions prepared only with WPI presented a slightly higher $d_{90,0}$ compared to emulsions prepared with WPI/LMWE, although the differences between WPI, WPI/Citrem and WPI/MoDi are not significant. The lowest $d_{90,0}$ was obtained for emulsions prepared with WPI/Lecithin. This is consistent with studies that showed that addition of lecithin to protein stabilized emulsions lead to smaller droplet sizes after homogenization (Shujie Wang et al., 2017). Viscosity values at a shear rate of 1000 s^{-1} are also presented in **Table IV-1**. As expected, no significant differences are observed between all emulsions. All emulsions presented a Newtonian behavior.

Table IV-1: Characteristics of feed emulsions and spray dried powders prepared using different emulsifier systems. For each characteristic, different letters indicate significant differences ($p < 0.05$).

	Emulsifier system			
Feed Emulsion	WPI	WPI/Lecithin	WPI/Citrem	WPI/MoDi
$d_{90,0}$ [µm]	3.99 ± 0.29[a]	2.49 ± 0.10[b]	3.34 ± 0.13[a]	3.50 ± 0.37[a]
Viscosity at 1000 s^{-1} [mPa·s]	33.1 ± 0.5[a]	32.3 ± 1.9[a]	31.0 ± 0.3[a]	31.56 ± 1.4[a]
Powders				
Moisture content [%]	2.81 ± 0.52[a]	2.69 ± 0.1[a]	2.15 ± 0.49[a]	2.46 ± 0.44[a]
Water activity []	0.23 ± 0.01[a]	0.21 ± 0.03[a]	0.20 ± 0.04[a]	0.23 ± 0.05[a]

SDSD during atomization are depicted in **Figure IV-1** at page 48. Emulsions stabilized with different emulsifier systems presented similar SDSD. This is expected as SDSD are dominated by emulsion viscosity and atomization conditions (e.g., nozzle type and pressure) (Lefebvre & Mcdonell, 2017). All these parameters were held constant for the different formulations. Similar spray droplet sizes indicate that the emulsions were subjected to similar stresses during atomization. Also, similar SDSD ensure that the surface area for heat and mass transfer was the same for all emulsions during the spray drying process. As the air temperature and volume flow were kept constant during spray drying, the same drying behavior is expected for all emulsions. These

results implicate that any differences observed in oil droplet size (see **chapter IV 3.2**) are due to different emulsifiers and not due to the drying process. Values of moisture content and water activities of the resulting powders were measured and are depicted in **Table IV-1**. As expected, no significant difference in the values are observed for the different emulsifier systems. Also, the values of moisture content and water activities are in a desirable industrial range to ensure product stability (Duckworth, 1975).

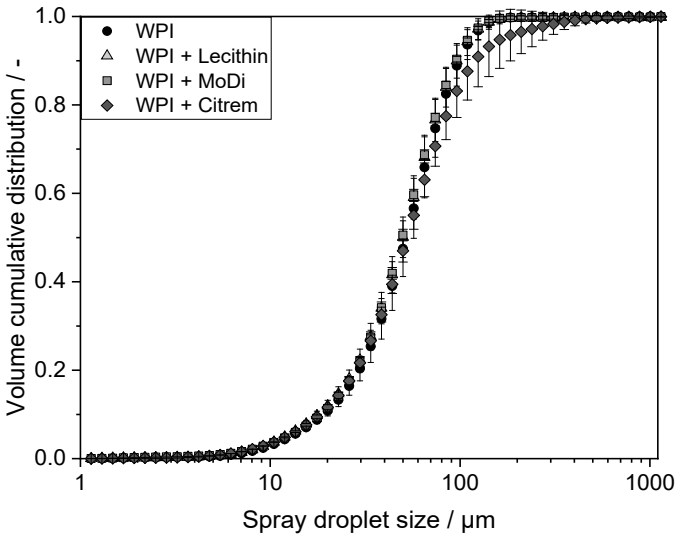

Figure IV-1. Droplet size distributions of spray droplets measured during atomization experiments with emulsions stabilized with whey protein isolate and WPI/LMWE.

IV.3.2 Oil droplet size after atomization and spray drying

The ODSD of the feed emulsions, the emulsions after atomization and the reconstituted emulsions after spray drying are depicted in **Figure IV-2** at page 49 for the different combinations of WPI/LMWE. In all cases the ODSD of the atomized emulsions (filled circles) is shifted toward lower values compared to their respective feed emulsions (filled triangles). These results indicate oil droplet breakup during atomization, which is consistent with previous studies (Taboada et al., 2020).

In the case of emulsions stabilized with WPI alone (**Figure IV-2** a), the ODSD of emulsions after atomization presents a bimodality. This bimodality is the result of oil droplet coalescence taking place during the atomization step, directly after droplet breakup (Taboada et al., 2020). When comparing the ODSD and the $d_{90,0}$ after atomization and after spray drying (**Table IV-2**, page 49), no significant differences are observed. These results indicate that the oil droplets were stable during the drying step for this emulsion.

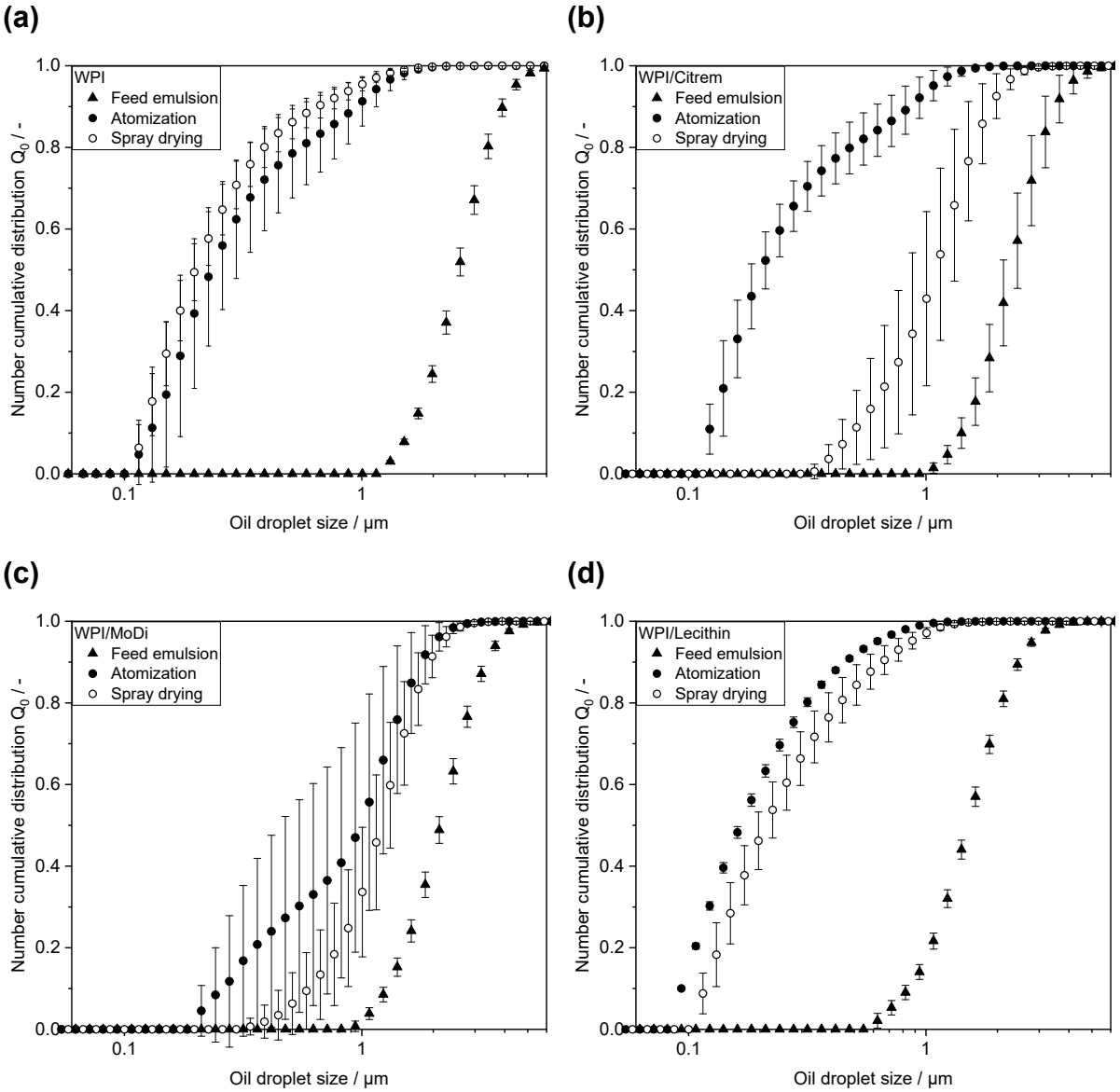

Figure IV-2: Number cumulative distributions of oil droplet size of emulsions stabilized with whey protein isolate and WPI/LMWE after atomization and spray drying. (a) whey protein isolate (b) WPI/Citrem (c) WPI/MoDi (d) WPI/Lecithin.

Table IV-2: Values of $d_{90,0}$ after atomization and after spray drying of emulsions stabilized with whey protein isolate (WPI) and WPI/LMWE. For each system, different letters indicate significant differences ($p < 0.05$).

Emulsifier system	$d_{90,0}$ after atomization [µm]	$d_{90,0}$ after spray drying [µm]
WPI	0.89 ± 0.28[a]	0.68 ± 0.14[a]
WPI/Lecithin	0.47 ± 0.02[a]	0.66 ± 0.11[b]
WPI/Citrem	0.86 ± 0.16[a]	1.83 ± 0.24[b]
WPI/MoDi	1.67 ± 0.35[a]	1.90 ± 0.17[a]

A different behavior is observed for emulsions stabilized with WPI/LMWE. In the case of emulsions stabilized with WPI/Citrem (**Figure IV-2** b, page 49), a bimodality is also observed in the ODSD of the emulsion after atomization, with a proportion of relatively small droplets (sizes between 0.1 µm and 0.3 µm) and larger droplets with sizes up to 1.1 µm. The bimodality is also the result of droplet coalescence during atomization (Taboada et al., 2020). The ODSD of the emulsion with WPI/Citrem after spray drying is shifted toward larger values, compared to the emulsion after atomization. In this case, oil droplet sizes start at 0.4 µm and range up to 2 µm. The value of $d_{90,0}$ after spray drying is significantly higher than the value after atomization (**Table IV-2**, page 49). These results indicate that coalescence of oil droplets takes place during the drying step with the system WPI/Citrem.

For emulsions stabilized with WPI/MoDi (**Figure IV-2** c, page 49), the ODSD after atomization also presents a bimodality, with a relatively small proportion of submicron droplets (sizes between 0.2 and 0.8 µm) and larger droplets with sizes up to 2 µm. Thus, the oil droplets after atomization are evidently larger compared to the oil droplets after atomization with the other emulsifier systems. In our previous study, we demonstrated that these large oil droplets are the result of droplet coalescence directly after oil droplet breakup during atomization (Taboada et al., 2020). When considering the ODSD of the emulsion after spray drying it can be seen that the proportion of small droplets is reduced compared to the ODSD after atomization, with the smallest oil droplets being around 0.4 µm. Both the ODSD after atomization and spray drying present large standard deviations. These large deviations are most probably a result of droplet coalescence, which is known to be a stochastic process (Neumann et al., 2018). Although the differences in $d_{90,0}$ after atomization and after spray drying are not significant (**Table IV-2**, page 49), the results on the ODSD suggest that the combination of WPI/MoDi further promotes coalescence during the drying step.

The results with the systems WPI/Citrem and WPI/MoDi suggest that addition of these LMWE is detrimental for oil droplet stabilization against coalescence during the drying step. Other studies have also reported increased oil droplet coalescence by addition of monoglycerides and fatty acid esters to protein stabilized emulsions (Danviriyakul et al., 2002; Drapala et al., 2017; Matsumiya et al., 2014). We can expect that during atomization and directly after oil droplet breakup, LMWE adsorb faster at the interface than whey protein (Bos & van Vliet, 2001). Once at the interface, competitive

adsorption with the protein may hinder the formation of the viscoelastic film at the interface (Bos & van Vliet, 2001), resulting in less stabilization against coalescence. Further details in interfacial mechanisms are explained in **chapter IV.3.3**

ODSD of emulsions after atomization and spray drying for the system with WPI/Lecithin are shown in **Figure IV-2** d at page 49. Differently to the other emulsifier systems of WPI/LMWE, the ODSD after atomization does not present a bimodality. In this case and as explained in our previous work, the combination WPI/lecithin prevents coalescence directly after oil droplet breakup during atomization (Taboada et al., 2020). Furthermore, the ODSD after spray drying is only slightly shifted to higher values and the distribution remains monomodal. In contrast with the emulsions with WPI/Citrem and WPI/Modi, oil droplets as small as 0.1 µm remain stable after spray drying in the emulsions with WPI/Lecithin. The results suggest that the oil droplets are well-protected against coalescence during the drying step with the combination WPI/Lecithin. An improved oil droplet stabilization by combination of whey proteins with lipid-based lecithin has also been reported in the literature (Bylaite et al., 2001; Shujie Wang et al., 2017).

IV.3.3 Interfacial tension and dilatational rheology influenced by LMWE

With the knowledge of interfacial tension and phase angle of dilatational rheology, we aim to explain the interfacial mechanisms which are affecting the oil drop size during the drying step of spray drying. The measured values of interfacial tension and phase angle for systems with β-LG and β-LG/LMWE are summarized in **Table IV-3** at page 52. The dominating proteins in WPI are β-LG and α-lactalbumin (Foegeding et al., 2011). The interfacial tension of β-LG was 15.3 ± 0.2 mN/m whereby a similar value was reported earlier for the same interfacial system (Keppler et al., 2021). The interfacial tension of 0.1% α-lactalbumin at pH 7 against oil was reported to be 15 mN/m as well (Lam & Nickerson, 2015). The values of interfacial tension (**Table IV-3**) are comparable with the values reported in our previous study for systems with WPI and WPI/LMWE (Taboada et al., 2020). Therefore, it is expected that the viscoelastic behavior of the systems reported in **chapter IV.3.2** is well modelled by the systems containing β-LG.

β-LG shows a viscoelastic behavior (**Table IV-3**) comparable to previous studies (Böttcher et al., 2017; Keppler et al., 2021). The phase angle of 6.9 ± 0.7° indicates a high elastic portion in the interfacial film. This viscoelastic behavior is expected to

increase the stability of emulsion droplets during processing steps (Bos & van Vliet, 2001; Lam & Nickerson, 2013). These results can explain the effects shown in (**Figure IV-2** a, page 49). Directly after oil droplet breakup during atomization there is some coalescence due to the slow kinetics of the protein (Lam & Nickerson, 2013). However, once the protein adsorbed at the interface, the highly elastic interfacial film protects the oil droplets against coalescence during the drying step. The high viscoelasticity is a result of high intermolecular interactions of protein molecules at the interface.

Table IV-3: Interfacial tension and phase angle of 0.1% β-LG with addition of 0.005% Lecithin, Citrem or MoDi at MCT-oil/water-interface after 14 h drop ripening and at 2.8% amplitude and 0.01 Hz.

	β-LG	**β-LG/Lecithin**	**β-LG/Citrem**	**β-LG/MoDi**
Interfacial tension [mN/m]	15.3 ± 0.2	8.4 ± 0.1	15.0 ± 0.2	15.2 ± 0.2
Phase angle [°]	6.9 ± 0.7	5.1 ± 0.5	9.5 ± 0.9	10.0 ± 1.0

In general, LMWE adsorb faster at the interface than proteins (Bos & van Vliet, 2001) and hinder the formation of the viscoelastic film (Wilde et al., 2004). For a system consisting of β-LG and citrem or MoDi, the interfacial tension barely changes compared to β-LG alone (**Table IV-3**) which is attributed to the comparatively low interfacial activity for citrem and MoDi as LMWE. The phase angle increases with addition of citrem and MoDi to around 10.0°. The increase in phase angle indicates a loss in elastic portion of the interfacial film. This loss in viscoelastic behavior was expected for an interfacial film with protein and LMWE and corresponds to previous literature (Wilde et al. 2004). By this, the increase of droplet size during the drying step with WPI/Citrem and WPI/Modi compared to the system with WPI alone can be explained. It is expected that directly after oil droplet breakup, the LMWE adsorbs fastly at the interface (Bos and van Vliet 2001) and hinder the formation of the viscoelastic film. Therefore, these films show less intermolecular interactions which results in an incomplete protection of the oil droplets against coalescence when forced in close contact during the drying step. The fewer interactions might be attributed to non-attractive interactions between the protein and citrem or MoDi. For a system containing citrem and β-LG, under neutral conditions both molecules are negatively charged due to the reported pka value and isoelectric point (Lam & Nickerson, 2013; Whitehurst, 2004). The repulsive forces between both molecules reduce the film elasticity which has been also reported by

(Wilde et al., 2004). Under neutral conditions, for the non-ionic MoDi, no attractive interactions to the protein are expected.

In comparison, the addition of lecithin lowers the interfacial tension and shifts the interfacial behavior to a more elastic response with a phase angle of 5.1 ± 0.5° (**Table IV-3**, page 52). This behavior can be explained by the high hydrophilic portion and thus high interfacial activity of the molecule (Murray & Dickinson, 1996; Whitehurst, 2004). The high interfacial activity of the lecithin molecule and the mutual high reduction in interfacial tension (**Table IV-3**) can explain that the smallest oil droplets were present in the feed emulsion (**Table IV-1**, page 47) and after atomization (**Table IV-2**, page 49). The high interfacial activity increases the elastic response by Gibbs-Marangoni-mechanisms (Murray & Dickinson, 1996; Wilde et al., 2004) which is attributed to the ability of lecithin to stabilize fastly unoccupied interfacial parts. This ability prevents coalescence of the oil droplets from the beginning of the spray drying process. The increased elastic behaviour of the film in the presence of lecithin makes the oil droplets less prone to coalescence when forced in close contact during drying. Synergetic effects between β-LG and several oil soluble LMWE, leading to higher interfacial stabilization have also been reported in the literature (Bylaite et al., 2001; Chen & Dickinson, 1995). The detailed mechanisms at the interface are not easy to predict due to the mixed molecular structure of LMWE. Also, the different time scales of the phenomena occurring during atomization and drying, and the presented measurements complicates the direct transfer of the observed effects. However, the results showed that LMWE and β-LG interact at the interface and lead to changes in the film viscoelasticity, even when the interfacial tension does not change. These effects go along with the observed coalescence of the oil droplets during the drying step. Therefore, the presented mechanisms give a better comprehension of the impact of interactions of emulsifiers on the changes of oil droplet size during spray drying.

IV.3.4 Powder particle size distributions and microstructure

Powder particle size distribution (PSD) after spray drying are depicted in **Figure IV-3** at page 54. Up to a value of around 100 µm, all powders presented very similar PSD. Only the PSD corresponding to the emulsion with WPI/Lecithin presented a monomodal distribution, with maximum values of around 200 µm. Powders with other emulsifier systems presented bimodal distributions and large particles up to 1,000 µm. As all emulsions presented the same SDSD during atomization (**Figure IV-1**, page 48),

and no values of spray droplet sizes close to 1,000 µm were measured, these large particle sizes cannot correspond to the primary size of the powder particles. These high values can only be explained by the formation of clumps or agglomerates in the powder, which were not destroyed by the dispersing gas during the measurements. From **Figure IV-3**, it is also noticeable that the powders with WPI/MoDi presented the largest particle sizes, followed by WPI/Citrem and WPI.

Powders clumps can also be detected in SEM micrographs (**Figure IV-4** , page 55). In agreement with the results shown in **Figure IV-3**, the largest clumps are observed in the case of the powders with WPI/MoDi (see circle in **Figure IV-4** c). Furthermore, dark areas corresponding to regions with free, non-encapsulated oil are also detected in all the powders (see arrows). It is well known that free surface oil can lead to the formation of liquid bridges between the particles (Nijdam & Langrish, 2006), leading to extensive clumping of the powders (Taneja et al., 2013). The amount of free surface oil has been previously correlated with coalescence of oil droplets during spray drying (Drapala et al., 2017; Drusch & Berg, 2008). With this knowledge it is obvious to assume that the systems with the most oil coalescence during the spray drying process (WPI/MoDi and WPI/Citrem) present the highest amount of non-encapsulated oil and have the highest tendency to clump formation. A detailed investigation on the free, non-encapsulated oil and the resulting storage characteristics of the investigated powders will be presented in a separate study.

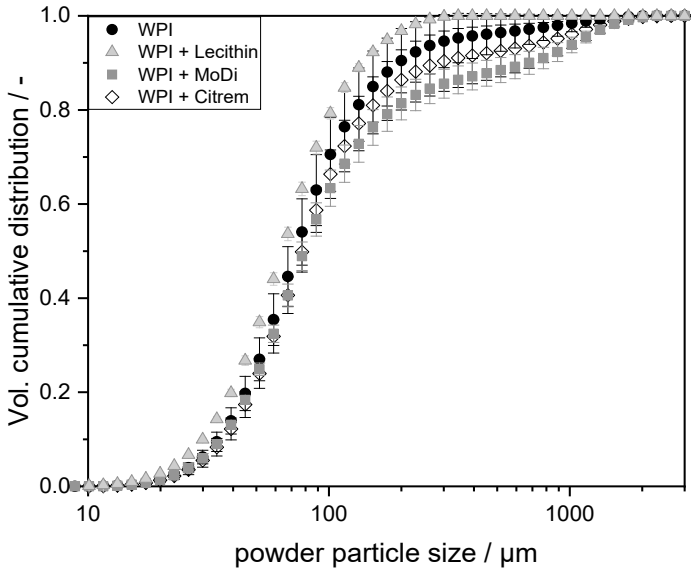

Figure IV-3. Particle size distributions of spray dried powders from emulsions stabilized with whey protein isolate and WPI/LMWE.

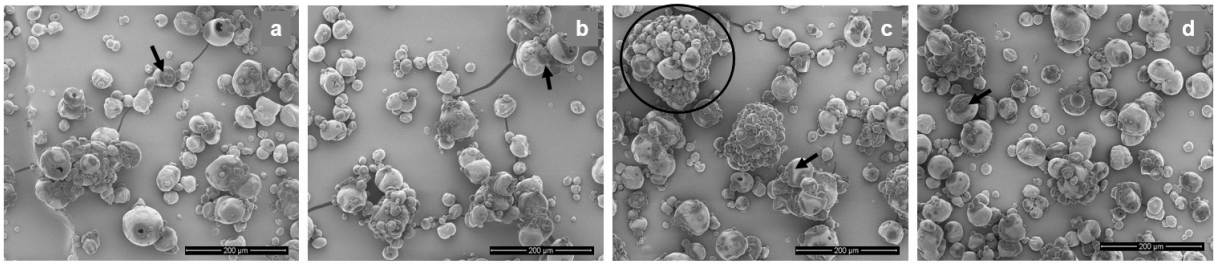

Figure IV-4. Scanning electron microscopy micrographs of spray dried emulsions stabilized with (a) whey protein isolate, (b) WPI/Lecithin, (c) WPI/MoDi, (d) WPI/Citrem. Magnification 500x.

IV.4 Conclusions

In the present study, the influence of addition of LMWE to WPI-stabilized emulsions on the changes of oil droplet size during the drying step of spray drying was investigated. No changes in ODSD after atomization and after spray drying were observed for emulsions stabilized with WPI. In the case of WPI/Lecithin, very small oil droplets remained stable after atomization and spray drying. The presence of lecithin seems to increase the stability of the interfacial film, making the oil droplets less prone to coalescence when forced in close contact during drying. These results go along with a lower interfacial tension and an increased elastic response of the interface with this system, as compared with protein alone. In contrast, emulsions with WPI/Citrem and WPI/MoDi presented an increase in oil droplet size during the drying step. A decrease in the elastic portion of the viscoelastic film by addition of these LMWE was observed. By this, the interfacial film of the oil droplets is less protected against coalescence when forced into close contact. Interestingly, significant differences in oil droplet coalescence and film viscoelasticity were observed between protein and mixed interfaces of protein with Citrem and MoDi, even when the interfacial tension was unchanged. By this, powders with significantly different characteristics, for example, clumping tendency, are obtained. The influence of the emulsifier system on the amount of free, non-encapsulated oil and on the storage stability of spray dried powders is currently being investigated. The results of this study are of high relevance to control the quality of whey/dairy-based food powder products. For an improved understanding of the effects, further studies are required in which a systematic approach is applied with LMWE of defined fatty acid and head group composition and so defined hydrophilic-lipophilic balance. Also, the effect of emulsifier concentrations should also be investigated.

V. Manuscript 3

Interfacial properties of β-lactoglobulin at the oil/water interface: influence of starch conversion products with varying dextrose equivalents

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Abstract

In spray dried emulsions, frequently milk proteins are used as interfacial active components and starch conversion products are added as matrix material at high concentrations. To characterize interfacial properties at the oil/water interface by commonly applied methods, low protein, and carbohydrate concentrations from 1 to 2% are usually analyzed. The impact of a higher concentration of starch conversion products was not investigated so far. Therefore, the formation and rheological properties of β-lactoglobulin (β-LG) stabilized films at the oil/water interface were investigated via short and long-time adsorption behavior using pendant drop tensiometry as well as dilatational and interfacial shear rheology. Suitability of the applied methods to the chosen samples with higher concentrations > 1-2% was verified by calculation of selected key numbers like capillary number and by detailed reviewing of the results which is summarized further on as key indicators.

It is hypothesized, that the increase in concentration via presence of starch conversion products will delay interfacial stabilization as a result of increased bulk viscosity with decreasing degree of degradation (dextrose equivalent) of the starch. Furthermore, this increase in concentration leads to more stable interfacial films due to thermodynamic incompatibility effects between protein and starch conversion products which results in increases of local protein concentration. Key indicators proved a general suitability of applied methods for the evaluation of the investigated samples. Moreover, results showed an increase in interfacial film stability and elastic properties alongside a decreased interfacial tension if starch conversion products were present in a high concentration.

Keywords: dilatational rheology, interfacial shear rheology, β-lactoglobulin, starch conversion product, excluded volume effect, application related concentration

Graphical abstract

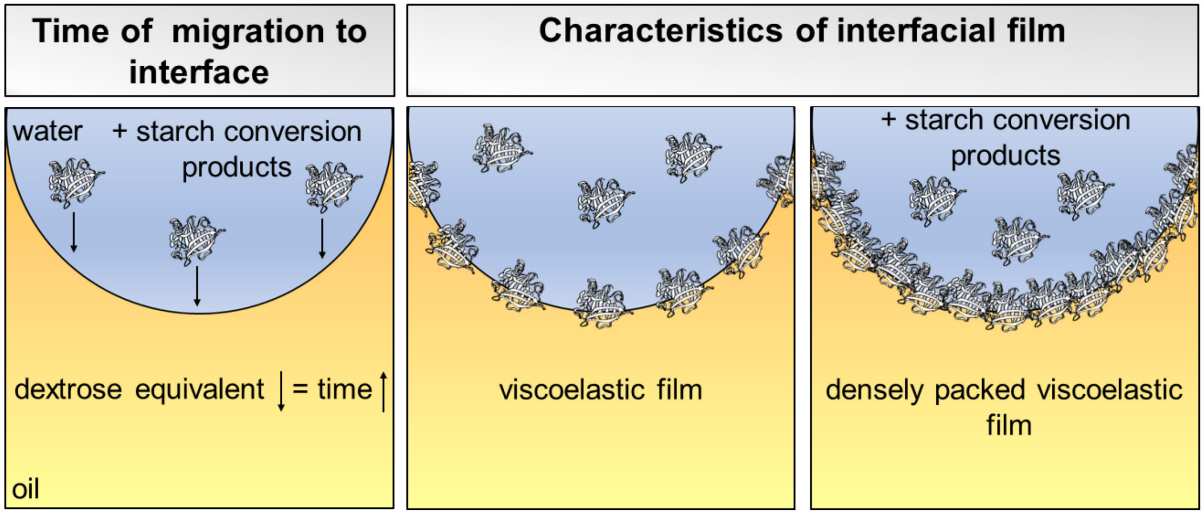

Figure V-1: Graphical Abstract: Interfacial properties of β-lactoglobulin at the oil/water interface: Influence of starch conversion products with varying dextrose equivalents.

V.1 Introduction

Oil in water emulsions are dispersed food systems that may be used in many different contexts. In emulsions high molecular weight emulsifiers like proteins may be present. Proteins lower the interfacial tension and stabilize the o/w interface (Lam & Nickerson, 2013). The process of interfacial stabilization by proteins can be divided into four stages: (1) protein migration through the bulk, (2) protein adsorption at the interface, (3) conformational reorganization of the protein and (4) formation of a stable interfacial film with intermolecular cross-linkings (Dickinson, 2011; Murray & Dickinson, 1996; Yampolskaya & Platikanov, 2006). Owing to its ability to provide stability to food emulsions β-LG is one of the most thoroughly investigated high molecular weight emulsifiers. Its interfacial properties were found to depend on several external factors like environmental conditions e.g. pH, temperature and ionic strength (Engelhardt et al., 2013; Jung et al., 2010; D. A. Kim et al., 2005; Moro et al., 2013; Roth et al., 2000; Rühs et al., 2012; Schestkowa et al., 2019).

In order to convert β-LG stabilized emulsions into powder, spray drying is a process commonly applied in the food industry. Starch conversion products are added to increase the dry matter content of the emulsions to 45% and above and to ensure the encapsulation of the oil drops in the emulsions. If proteins and polysaccharides are present in an aqueous system, different thermodynamic phenomena may occur, either co-solubility or incompatibility resulting in complexation or phase separation (de Kruif & Tuinier, 2001). Co-solubility is represented by coexisting molecules while the incompatibility occurs as phase segregation of the two molecules and the complexation of proteins and polysaccharides is reflected in an associative behavior between both of them (de Kruif & Tuinier, 2001). These thermodynamic mechanisms within the bulk phase will affect the resulting interfacial properties. As a result from the incompatibility between protein and polysaccharide, Rodriguez Patino and Pilosof (2011) assumed a film with a higher protein load at the interface (Rodríguez Patino & Pilosof, 2011). This incompatibility, resulting from the excluded volume effect, was also verified by Antipova and Semenova (1997) using a light scattering method (Antipova & Semenova, 1997).

Since Antipova and Semenova (1997), Baeza et al. (2004), Baeza et al. (2005) and Perez et al. (2010) observed that the interfacial tension was dependent on the type of proteins and polysaccharides, it can be assumed that the molecular structure of the starch conversion products will affect the extent of the described effects (Antipova &

Semenova, 1997; Baeza et al., 2004, 2005; Perez et al., 2010). In addition, the molecular structure of the starch conversion products commonly known as dextrose equivalent (DE) will influence the viscosity of the bulk phase (Dokic et al., 1998). This should in turn influence the diffusion based short time adsorption transport to the interface, as described in the Stokes-Einstein equation as one part of the Ward-Tordai adsorption theory (Ward & Tordai, 1946).

Typically, adsorption kinetics and interfacial properties are investigated via drop tensiometry and interfacial shear rheology. In this context, the former can be used to characterize the adsorption to the interface at different time scales as well as the impact of expansion and compression on the characteristics of the interfacial film, while the latter describes the interactions within the interfacial film (Krägel et al., 2008; Lucassen-Reynders, 1993; Tamm et al., 2012). Both methods are typically used to characterize interfacial properties of substances under aqueous, highly diluted conditions (e.g. (Miller et al., 2010; Rühs et al., 2012; Schestkowa et al., 2019)). However, it can be reasonably assumed that changes in physical values like bulk viscosity, flow behavior or density will affect the interfacial properties. In addition, Bertsch and Fischer (2019 and 2020) and Bertsch et al. (2018, 2020) showed that bulk viscosity increase by gelation superimposed to interfacial stabilization can result in misinterpretation of interfacial shear rheological results (Bertsch et al., 2018, 2020; Bertsch & Fischer, 2019, 2020). So far, no study systematically analyzed the applicability of the interfacial methods at an increased concentration far beyond the common concentrations of 1-2%. However, the suitability of the measurement equipment as well as the model-based calculations can be verified via specific indicators for accuracy of measurement and calculation (Berry et al., 2015; Erni et al., 2003; Freer et al., 2005; Lee et al., 1991; Loglio et al., 2004).

Therefore, the aim of this study is to evaluate the suitability of drop tensiometry and interfacial shear rheology for the characterization of film formation and interfacial properties at an oil/water interface in systems with a dry matter content of 35% in the water phase. Moreover, the influence of different degrees of degradation in the starch conversion products should also be investigated.

It is assumed that accurate results of interfacial rheological methods can be gained at a high dry matter content in the water phase for Newtonian fluids if the effect of changes in sample characteristics in viscosity and density on interfacial measurement and

derived results can be controlled. For drop tensiometry measurements an appropriate balance of interfacial tension and gravitational forces in dependence on drop volume and needle diameter needs to be maintained for accurate Young Laplace fitting. A prerequisite for dilatational rheology is the prevention of critical and droplet deforming capillary forces. For interfacial shear rheology, minimal motion of the subphase needs to be guaranteed. These prerequisites for accurate interfacial rheological methods are met by careful consideration of the following key numbers: Bond and Worthington number, capillary number and Boussinesq number.

Furthermore, it is hypothesized, that the increase in dry matter content in the water phase via addition of starch conversion products will delay interfacial stabilization as a result of increased bulk viscosity with decreasing dextrose equivalent (DE). The high dry matter content in the water phase will further lead to lower interfacial tension and more stable interfacial films with an increase in intermolecular interactions and elastic response of the viscoelastic β-LG-film due to the increase in local protein concentration caused by thermodynamic incompatibility effects between protein and starch conversion products. Therefore, pendant drop analysis with a two-fluid needle is used to characterize the adsorption behavior. The interfacial film is characterized in its viscoelasticity and strength of its intermolecular network via response of the interfacial area to expansion and compression by dilatational rheology and via nondestructive oscillation by interfacial shear rheology, respectively (Krägel et al., 2008; Lucassen-Reynders, 1993).

V.2 Materials and methods

β-LG was isolated from whey protein isolate (Bipro, Agropur Dairy Cooperative Inc., Minnesota, USA) with a method described elsewhere (Keppler et al., 2014). The resulting protein powder has a dry matter of 90.7 ± 1.0% and a protein content of 90.1 ± 1.2%, while the protein content is composed of 98.11% isolated β-LG, 0.37% α-lactalbumin and 1.51% denaturated β-LG (analyzed according to Keppler et al. 2014). Medium-chain-triglyceride oil (MCT-oil) WITARIX® MCT 60/40 was kindly provided from IOI Oleo GmbH (Hamburg, Germany). Interfacial active substances in the MCT-oil were removed via magnesium silicate adsorption (Florisil®, Carl Roth GmbH, Karlsruhe Germany). Glucose syrup with a DE of 37.3 (GS 37, C*Dry ™ GL 01934), maltodextrin with a DE of 13.9 (MD 14, C*Dry ™ MD 01910) and maltodextrin with a DE of 8.8 (MD 9, C*Dry ™ MD 01958) were purchased from Cargill Deutschland GmbH (Krefeld, Germany). The starch conversion products differ in their dextrose equivalent that means their hydrolyzation grade of starch. The dextrose equivalent is a measure of the reducing power of the starch which is calculated as dextrose and expressed as percentage of dry matter (Blanchard & Katz, 2006). All starch conversion products had negligible protein residues (0.1-0.15%, measured with DUMATHERM, C. Gerhardt GmbH & Co. KG, Königswinter, Germany). Distilled water was used for all experiments.

V.2.1 Molecular characterization of starch conversion products by means of size exclusion chromatography - multi angle light scattering - differential refractive index (SEC-MALS-DRI)

Aqueous solutions of the starch conversion products were prepared by dissolving in water to a concentration 2.5 d.m.%. The solutions were diluted 1:10 (v/v) in dimethyl sulfoxide (DMSO) preheated at 40 °C to a concentration of about 2.5 mg/mL and passed through PTFE filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) before analysis.

The molecular characterization was carried out by means of SEC-MALS-DRI as described elsewhere (Ulbrich, Daler, et al., 2019). The separation was executed with a SEC-3010 module (WGE Dr. Bures GmbH & Co. KG, Dallgow-Doeberitz, Germany) including degasser, pump and auto sampler connected to a MALS detector (Bi-MwA, Brookhaven Instruments Corporation, Holtsville, NY, USA) and a differential refractive index detector (DRI). The samples were eluted with degassed DMSO (Carl Roth GmbH

& Co. KG, Karlsruhe, Germany) containing 0.1 M $NaNO_3$ at a flow rate of 0.5 mL/min and a temperature of 70 °C. Data were collected and processed using ParSEC Enhanced V5.61 chromatography software to give the concentration of the eluted solution at each retention volume (SEC chromatograms). This method provides no differentiation between branched and linear molecule structures since the separation is according to the hydrodynamic volume. Therefore, the results indicate mainly the molecular size of the starch conversion products.

V.2.2 Preparation of protein and protein/starch conversion product-solutions

For dilatational and interfacial shear rheological measurements protein- and protein/starch conversion product-solutions were prepared. β-LG was dissolved and stirred in distilled water for approximately 2 h and reached a pH around 7. The starch conversion product was pre-solubilized in distilled water with usage of a stirring device (RCT Basic, IKA-Werke GmbH & Co. KG) for approximately 2 h and the pH was adjusted to 7 with 1M NaOH. Both solutions were combined to obtain concentrations of 0.1% protein and 34.9 d.m.% starch conversion product, were stirred for further 3 h and were stored at 5 °C for about 14 h overnight. Afterwards, all solutions were stirred to obtain a homogenous solution before measurement. The pH was adjusted if necessary.

For the adsorption behavior measurement, separate protein and starch conversion product solutions were prepared in the same way as described above with a protein concentration of 1.5% and a starch conversion product concentration of 15.96%. Both solutions are combined in a particular ratio in a two-fluid needle to reach a protein concentration of 0.1% and a starch conversion product concentration of 14.9%. Here, a lower starch conversion product concentration was used due to limited file size and recording time of the video.

V.2.3 Characterization of physical values

All solutions were characterized in physical values - viscosity and density. Viscosity was determined with a flow plot from 0.1-1000 1/s under usage of a cylinder (double gap, DG 26.7), a Physica MCR 102 and MCR 501 rheometer (Anton Paar GmbH, Ostfildern, Germany). Density was determined with an oscillating U-tube (DMA 35, Chempro/Paar GmbH). The experiments were performed in triplicate.

V.2.4 Time dependent adsorption behavior

Time dependent adsorption behavior was measured by pendant drop tensiometry (OCA-20, Dataphysics Instruments GmbH, Filderstadt, Germany) at 22 °C. A high speed camera is used to record the drop shape. The improved Young Laplace equation (1) is fitted to the drop curvature of the drop profile while the curvature is defined with the changing tangent angle to the length at the drop curvature. With computational calculation the interfacial tension is determined.

$$\frac{d\varphi}{ds} = 2 - \frac{\Delta\rho g R_0^2 z}{\gamma} - \frac{sin\varphi}{r} \quad (1)$$

The equation (1) takes account of the change in tangent angle (dφ) to the change in length (ds) at the drop curvature, the cylindrical drop coordinates (r and z) and the radius of curvature at the drop apex (R_0). These parameters are crucial to analyze the interfacial tension out of the drop shape. Furthermore, the density of water and oil phase (Δρ) and the gravity constant (g) are taken into account as drop shape influencing factors (Berry et al., 2015).

The Bond number and Worthington number are indicators for an accurate Young Laplace fitting and focus on the drop shape and the drop volume, respectively (Berry et al., 2015). The Bond number is a part of the Young Laplace equation and estimates the drop shape for ideal Young Laplace fitting (equation 2 (Berry et al., 2015)). If the Bond number is too small (<< 0.15), the drop approaches a spherical shape, and the Young Laplace fitting is inaccurate [28].

$$Bo = \frac{\Delta\rho g R_0^2}{\gamma} \quad (2)$$

$$Wo = \frac{V_d}{V_{max}} \quad (3) \text{ with}$$

$$V_{max} = \frac{\pi D_n \gamma}{\Delta\rho g} \quad (4)$$

The Worthington number depends on the maximal drop volume (V_{max}) and the used drop volume (V_d) (equation 3, (Berry et al., 2015)). V_{max} was defined by Harkins & Brown (1919) (Harkins & Brown, 1919) and depends on the needle diameter (D_n = 2 mm), the interfacial tension (γ), the density difference (Δ_ρ) and the gravimetric constant (g) (equation 4). This number can be used to find an appropriate drop volume

to a given needle diameter for accurate Young Laplace fitting and should be very close to 1 (Berry et al., 2015).

Within short time adsorption behavior, a two fluid-needle was used to investigate the transport time of β-LG from the injection point to the oil/water interface (lag time) and the interfacial pressure after a certain time. A drop with water or with a solution of starch conversion products with 42 ± 0.1 µL was formed and 3 ± 0.1 µL of a solution of β-LG was injected to reach a protein concentration of 0.1% within the drop. The experiments were performed in triplicate. On the basis of Böttcher, Keppler, and Drusch (2017) and Schestkowa et al. (2019) lag time was determined from the injection time point to the start point of decreasing interfacial tension (Böttcher et al., 2017; Schestkowa et al., 2019). The analysis is influenced by injection induced motion with a low velocity field in the bulk phase. The lag time is not only diffusion based.

Long term adsorption behavior was measured by drop tensiometry (PAT1M, Sinterface Technologies e.K., Berlin, Germany) with a single needle at 22 °C. The interfacial tension was recorded for 14 h. The experiments were performed in triplicate.

V.2.5 Dilatational rheology

Dilatational rheology was investigated by pendant drop tensiometry (PAT1M, Sinterface Technologies e.K., Berlin, Germany) at 22 °C. A high speed camera is used to record the drop shape during sinusoidal oscillation. All previously discussed key indicators (2-4) are still relevant. Furthermore, the interfacial tension and the drop area are recorded and used to calculate the complex dilatational modulus (Lucassen-Reynders, 1993) with Fourier analysis (equation 5). This equation calculates the proportion of the change in interfacial tension (σ) and area (A).

$$E^* = \frac{d\sigma}{d \ln A} \quad (5)$$

An indicator of accuracy of the Fourier analysis is the harmonic distortion. The excitation-response behavior of the drop needs to be in linearity for a harmonic behavior. If the relationship is non-linear, the system shows non-harmonic distortion which can result in severe a calculation mistake (Loglio et al., 2004). The harmonic distortion was observed. All data represent a well-developed sinusoidal shape without non-harmonic distortion.

The accuracy of the dilatational measurement can be furthermore estimated with the capillary number (Ca, equation 6). This number is an indicator for the frequency or amplitude limits (Freer et al., 2005) and depends on the bulk Newtonian viscosity of the drop and the surrounding liquid (Δμ), the oscillation frequency (ω), the amplitude of volume oscillation (ΔV), the interfacial tension of the system (γ) and the capillary radius (a) (Freer et al., 2005). The capillary number has to be << 1 to avoid viscous forces which might deform the drop and cause an inaccuracy in dilatational result calculation (Freer et al., 2005). Furthermore, it is stated that Ca should be ideally < 0.002 to avoid any inaccuracy (Freer et al., 2005; Ravera et al., 2010).

$$Ca = \frac{\Delta\mu\omega\Delta V}{\gamma a^2} \quad (6)$$

During the dilatational rheology, a protein or protein/ starch conversion product solution drop of 30 mm^3 was formed in MCT-oil with a viscosity of 30 mPas. The drop was equilibrated for 14 h (see long term adsorption **chapter V 2.4**). Afterwards, a frequency sweep (2.8% amplitude, 0.001 to 0.1 Hz) followed by an amplitude sweep (0.01 Hz, 0.7% to 7% amplitude) were performed. The experiments were performed in triplicate. The dilatational modulus E* is calculated by the change in interfacial tension and the simultaneous change in drop area during sinusoidal expansion and compression within the sweeps. The elastic modulus (E') and viscous modulus (E'') are determined out of E* (see equation 7) (Lucassen-Reynders, 1993). The phase angle ($\boldsymbol{\phi}$) between the sinusoidal curves of interfacial tension and drop area is calculated with tan ($\boldsymbol{\phi}$) = E''/E'. If both curves are in phase, the interfacial film reacts only elastic. If there is a phase shift of 90° between the curves, the interfacial film reacts only viscous. A value between 0° and 90° shows a viscoelastic behavior of the film.

$$E^* = E_d + i\omega\eta_d = \ E' + iE'' \quad (7)$$

Lissajous-plots give further details in the viscoelastic behavior of interfacial films. These figures are plotted with the change in interfacial tension ($\Delta IFT = \sigma - \sigma_0$) versus the change in area ($\Delta A/A_0$; $\Delta A = A - A_0$). σ_0 and A_0 represent the interfacial tension and area at zero strain.

V.2.6 Interfacial shear rheology

Interfacial shear rheology was performed with a Physica MCR301 und MCR102 rheometer (Anton Paar Germany GmbH, Ostfildern, Germany) provided with an

interfacial bicone (Bicone, Bi-C68-5, Anton Paar Germany GmbH, Ostfildern, Germany) at 20 °C. The software-based calculations are based on records of interfacial angular velocity distributions and on the complex viscosity surface fluid model. According to the model requirements, the water and oil phase need to be Newtonian and the density, the viscosity of both phases as well as the cell and bicone geometric data are taken into account (Lee et al., 1991).The Boussinesq number (8) is an indicator of the bulk phase undesirable movement. The interfacial viscosity (η), the oil viscosity (η_o) and the protein or protein/starch conversion product solution viscosity ($\eta_{p/c}$) as well as the measurement cell radius (R) are considered for the calculations of the Boussinesq number (Erni et al., 2003). If the Boussinesq number is higher than 1, the bicone induced movement is situated at the interface and not in the surrounding water and oil bulk phase (Rühs et al., 2012). However, the software automatically corrects the subphase drag for high and low Boussinesq numbers. Thus, this number will not be discussed in detail. More details about operating windows for oscillatory interfacial shear rheology can be found in other publications (Renggli et al., 2020; Tajuelo & Rubio, 2018).

$$Bou = \frac{\eta}{(\eta_o + \eta_{p/c})R} \quad (8)$$

During interfacial shear rheological measurements, the protein or protein/starch conversion product solution was carefully poured into the interfacial shear glass cylinder with the help of a glass rod. Bubbles were gently and immediately removed with pasteur pipettes. The bicone was positioned directly at the interface and covered with purified MCT-oil. The interfacial film development was monitored for 23 h at 1 Hz and 0.1% amplitude. Afterwards the film was investigated via frequency sweep (0.1% amplitude; 1 - 0.001 Hz) followed by amplitude sweep (0.3 Hz; 0.01% - 100% amplitude). The experiments were performed in triplicate. For comparison of the amplitude sweeps, the intersection points of G'=G'' were calculated with RHEOPLUS/32 Multi6 V3.62 (Anton Paar GmbH, Ostfildern, Germany). The complex shear modulus (G*), the elastic modulus (G') and the viscous modulus (G'') are defined in a similar way as the dilatational moduli.

V.2.7 Statistical analysis

Statistical analysis was performed by univariate ANOVA with significance measured by post-hoc Scheffé test ($p < 0.05$).

V.3 Results and Discussion

V.3.1 Molecular composition of starch conversion products

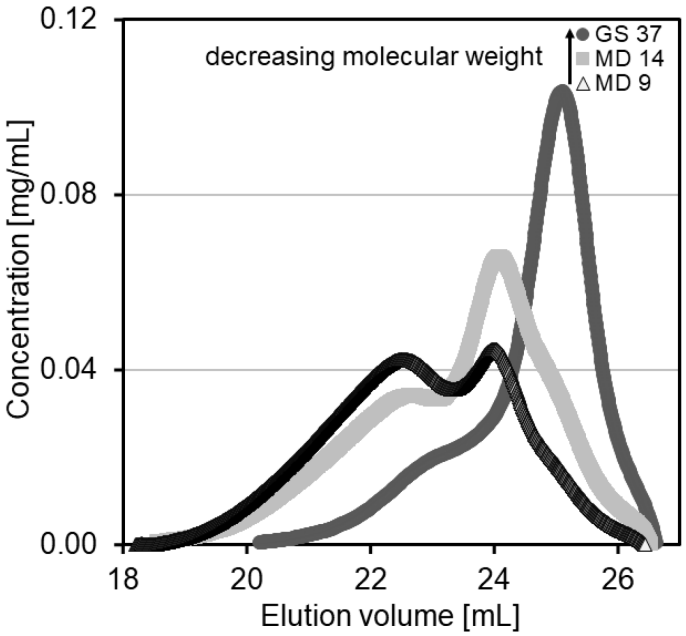

Figure V-2: SEC chromatogram of maltodextrin DE 9 (MD 9), 14 (MD 14) and glucose syrup DE 37 (GS 37).

Figure V-2 displays the chromatograms of the starch conversion products determined by means of SEC-MALS-DRI. The chromatograms of MD 14 and MD 9 are similar in terms of shape. Two main fractions can be distinguished between about 18 and 23 mL elution volume and about 23 and 26 mL reflecting different molecular size fractions. However, the relative portions differ strongly depending on the degree of molecular degradation. Increasing DE of the maltodextrin shifted the chromatogram to higher elution volume, indicating higher degree of degradation. Moreover, the ratio of the fractions changed remarkably. The chromatogram of GS 37 was distinct from the respective maltodextrin samples in terms of shape and position (elution volume range). In particular the chromatogram area between 24 and 26.5 mL relates to the maltose-rich (24-26 mL) and glucose-rich fractions (25-26.5 mL) (Ulbrich, Terstegen, et al., 2019). Compared to the maltodextrins, the glucose syrup has a considerably higher mono- and di-saccharide content as expected.

V.3.2 Physical values of solutions

Density and viscosity are recorded for all solutions (**Table V-1**, page 69). The viscosity of the solutions increases with decreasing DE while the solutions show Newtonian

behavior. This increase was differently pronounced in samples used for short time adsorption and interfacial rheology experiments owing to their different total starch conversion product concentrations. As expected, lower concentrations led to lower viscosities but also to a less pronounced increase in viscosity with decreasing DE (1.1 ± 0.0 mPas for β-LG to 5.4 ± 0.0 mPas for MD 9). In contrast at 35% concentration of protein and starch conversion products the viscosities ranged from 1.1 ± 0.1 mPas (β-LG) to 78.6 ± 2.5 mPas (MD 9). In earlier studies, it was shown that the viscosity is increasing in an exponential way especially for low DE with increasing concentration (Dokic et al., 1998).

Table V-1: Physical values of protein and protein/starch conversion product solutions.

Sample	Viscosity [mPas]	Density [g/cm³]	Sample	Viscosity [mPas]	Density [g/cm³]
0.1% β-LG	1.1 ± 0.1 [d]	1.00 ± 0.00 [b]	**1.5% β-LG**	1.1 ± 0.0[d]	1.00 ± 0.00[b]
+ 34.9% MD 9	78.6 ± 2.5[a]	1.16 ± 0.00 [a]	**15.96% MD 9**	5.4 ± 0.0 [a]	1.06 ± 0.00[a]
+ 34.9% MD 14	35.5 ± 0.8[b]	1.16 ± 0.00 [a]	**15.96% MD 14**	3.6 ± 0.0 [b]	1.06 ± 0.00[a]
+ 34.9% GS 37	7.9 ± 0.1 [c]	1.15 ± 0.00 [a]	**15.96% GS 37**	2.1 ± 0.0 [c]	1.06 ± 0.00[a]

letters a-c indicate significant differences for all columns ($p < 0.05$).

The density of a pure 0.1% or 1.5% β-LG solution was 1 g/cm³. With addition of 34.9% starch conversion products, the density increased to 1.15 or 1.16 g/cm³. All starch conversion product solutions with 15.95% showed a density of 1.06 g/cm³ (**Table V-1**).

V.3.3 Calculation and interpretation of key indicators for the evaluation of method suitability

Precision of the determination of interfacial tension can be estimated with the Bond and Worthington number (Berry et al., 2015). The Bond number (equation 2) was calculated for the protein/starch conversion product solutions with a density difference of 0.21 g/cm³, an interfacial tension of 12 mN/m and a radius of approx. 1.93 mm which yields in a number of 0.64. Thus, the value of the Bond number lies above the critical value of 0.15. The calculated value of the Worthington number is with 0.82 close to the critical value of 1 (equation 3). Furthermore, with volume increase of maximal 5 mm³ during oscillation the Worthington number will not be beyond the critical value.

Therefore, Bond and Worthington number indicate an accurate determination of the interfacial tension in presence of starch conversion products at high concentrations.

For the dilatational rheology, the harmonic distortion and the capillary number are indicators for accurate measurements. All data showed harmonic distortion. The frequency limit for liquid-liquid interfaces was earlier stated to be 0.1 Hz (Ravera et al., 2010). Moreover, Freer et al. (2005) suggested the critical capillary number at $Ca < 0.002$ to neglect viscous forces (Freer et al., 2005). This criterion applied for all investigated samples, amplitudes, and frequencies except for presence of MD 9 at 0.1 Hz and 2.8% amplitude which reached its maximum at $Ca = 0.0035$. Therefore, the affiliated data can be described as robust except for MD 9 at 0.1 Hz and 2.8% amplitude. Another indicator for amplitude limits in the dilatational measurement is a partially disrupted compression. A high viscosity of the solution can hinder the full transfer of the downward movement of the piston in the drop tensiometer to decrease the drop volume. In this study the compression was partially disrupted for viscosities from 35 mPas and amplitudes above 4.2%. Above amplitudes of 4.2%, during compression the target amplitude was not reached occasionally. However, in interfacial tension-volume/area-time-graphs provided by the software measurements as well as target values can be controlled, and incorrect measurements can be excluded. We recommend avoiding these problems by adjustment of the pendant drop tensiometer equipment, for instance the capillary diameter and the pump performance hence pump volume.

The interfacial shear rheology is not susceptible to changes in physical characteristics like bulk viscosity, flow behavior or density. All these physical values are considered within the software based calculations (Lee et al., 1991). Therefore, evaluation of interfacial properties of β-LG with presence of high dry matter content of 35% in the water phase is feasible with some limitations. Limitations might occur within the dilatational rheology for high amplitudes above 4.2%, and high frequencies of 0.1 Hz for highly viscous solutions (80 mPas). In general, indicators for measurement issues are a non-harmonic distortion of the drop, a partially disrupted compression, and a high capillary number. These indicators need to be observed critically during and after the measurement. Especially for a high capillary number and a disrupted compression, the data do not represent the reaction of the interfacial film on the target stress and should be excluded.

V.3.4 Time dependent adsorption behavior

The first stage of the interfacial stabilization—migration of the protein to the interface—may be characterized via lag time and interfacial pressure after a defined time. Short time adsorption experiments have been performed with a two-fluid needle. The influence of starch conversion products with different DE on β-LG adsorption behavior is shown in **Figure V-3**. A significant increase in lag time with reduced DE reflecting an increased viscosity becomes obvious (**Figure V-3** a). The increase in viscosity due to the lowering degree of degradation of the starch conversion products slows the translational motion of the protein to the interface. This motion is affected by the injection induced motion of the fluid which is influenced by the bulk viscosity according to the Navier-Stokes equation. However, comparison of our determined β-LG migration time with other literature is difficult due to the multitude of methods and influencing factors. Schestkowa et al. (2019) and Böttcher et al. (2017) estimated a transportation time of around 5 s for 0.1% β-LG to the o/w interface with the same equipment (Böttcher et al., 2017; Schestkowa et al., 2019). This increase of 4 s can be explained with their lower protein injection volume and lower water drop volume which causes less bulk motion. In comparison, these experimental conditions result in a lower velocity field than in our case.

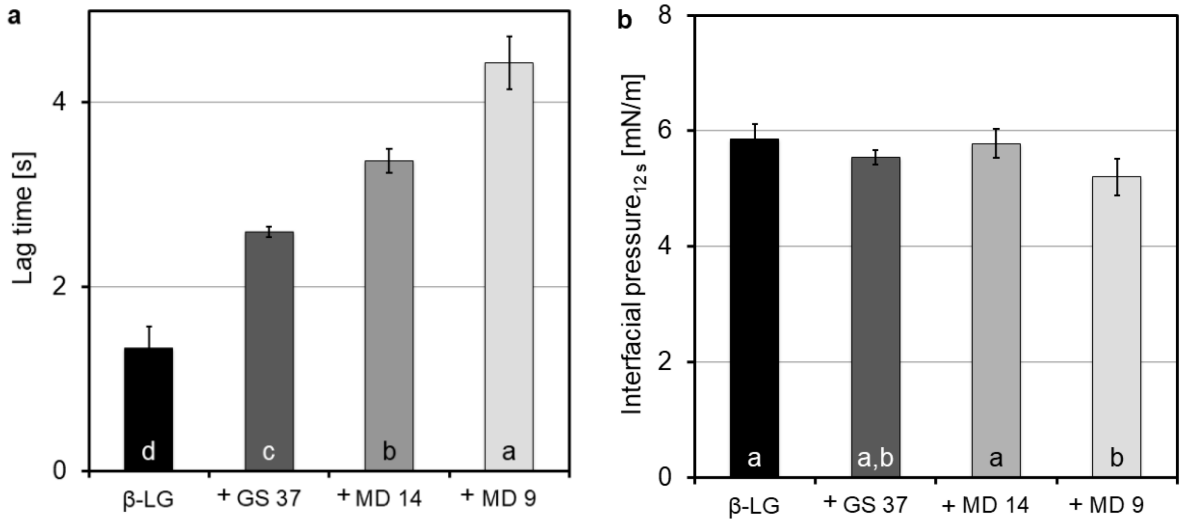

Figure V-3: a) Lag time and b) interfacial pressure 12 s after injection of 0.1% β-LG with presence of 14.9% glucose syrup (DE 37) and maltodextrin (DE 14 and 9) at MCT-oil/water-interface, letters a-d indicate significant differences ($p < 0.05$).

Subsequently, the short time protein adsorption at the interface as second stage takes place. 12 s after protein injection, the interfacial pressure is similar for all samples and only β- LG in water and β-LG in MD 9 show a significant difference (**Figure V-3** b). Within such a short time, the results might be influenced by the velocity field due to protein injection and the lower total starch conversion product concentration. Thermodynamic effects between protein and polysaccharides cannot be discussed on the basis of the results of short time adsorption.

In long term adsorption studies after 14 h of equilibrating, interfacial tension was lower in samples with higher starch conversion product concentration, reduced degradation level and increasing viscosity (**Figure V-4**, page 73). Differences between MD 14 and MD 9 were not significant. Baeza et al (2004) and Baeza et al. (2005) ascribed the reduction of surface tension to thermodynamic incompatibility of β-LG and several neutral polysaccharides (Baeza et al., 2004, 2005). The underlying mechanisms are more noticeable due to ongoing conformational reorganization of the protein and the development of intermolecular interactions during stage 3 and 4 of interfacial stabilization, which in turn lead to phase separation with time. Perez et al. (2010) showed the same effect of decreased interfacial tension of β-LG at pH 7 with addition of xanthan (Perez et al., 2010). An increased tendency of incompatibility was described for globulins and neutral polysaccharide mixtures at a pH above the isoelectric point (pI) and high concentrations for both substances (Grinberg & Tolstoguzov, 1997). This thermodynamic incompatibility accompanies the local protein enrichment at the interface (Rodríguez Patino & Pilosof, 2011).

When β-Lg is the only component within the water phase of the emulsion, it tends to form intermolecular β-sheets at the interface (Schestkowa et al., 2020). It is assumed that the interfacial film with protein enrichment is densely packed with intermolecular β-sheets connecting the β-Lg molecules. Furthermore, the protein concentration around the interface is higher due to the protein enrichment. We therefore propose that a reduced interfacial tension is a result of the excluded volume effect (Antipova & Semenova, 1997). A correlation between reduced surface tension and the excluded volume effect was indicated by second virial coefficient (Antipova & Semenova, 1997).

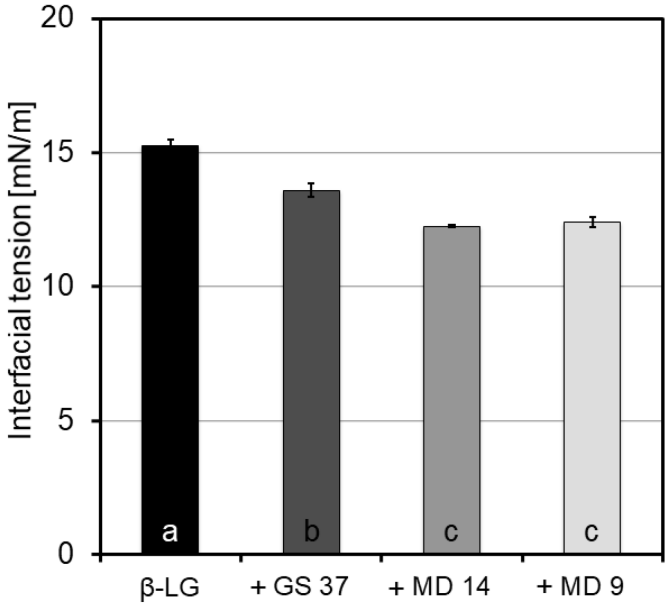

Figure V-4: Interfacial tension of 0.1% β-LG with presence of 34.9% glucose syrup (DE 37) and maltodextrin (DE 14 and DE 9) at MCT-oil/ water-interface after 14 h drop ripening, letters a-c indicates significant differences ($p < 0.05$).

In addition, the decrease in interfacial tension was even more pronounced with decreasing dextrose equivalent and consequently decreasing level of degradation (**Figure V-4**). This can be mainly explained by the increase in molecular size which enhances thermodynamic incompatibility for MD 14 and MD 9 (Semenova & Dickinson, 2010). With increasing level of degradation, the proportion of mono- and disaccharides increases for GS 37 (**Figure V-2**, page 68). The role of mono- and disaccharides as conformational stabilizers of proteins was reported to be a result of steric exclusion to proteins, cohesive forces of mono- and disaccharides, and intra-molecular protein interactions as driving forces of clustering (Shukla et al., 2011). It was shown for a globular protein (lysozyme) that its hydration increased with increasing sugar concentration (Lerbret et al., 2007). It can be assumed that for the glucose syrup the higher proportion of mono- and disaccharides compared to maltodextrins would result in a slightly lower protein enrichment and reduced thermodynamic incompatibility (Antipova & Semenova, 1995).

V.3.5 Dilatational rheology

The dilatational rheology is used to investigate the viscoelastic response of an equilibrated interfacial film to expansion and compression. The equilibrated film is characterized with a frequency sweep followed by an amplitude sweep. Within the

frequency sweep, the β-LG-film shows an increase of the elastic modulus with higher frequencies (**Figure V-5**, page 75). With presence of starch conversion products, the elastic modulus remains nearly constant. At frequencies between 0.02 to 0.1 Hz, the elastic modulus is lower than for the pure β-LG. The viscous modulus decreases with presence of starch conversion products with no frequency dependence. Therefore, the typical viscoelastic behavior of β-LG is shifted to a more elastic response for all frequencies without difference in starch conversion products' DE (Lissajous-plots in **Figure V-5**). Furthermore, the phase angle for β- LG at 0.01 Hz is reduced with presence of starch conversion products with decreasing degradation level from 12.33° ± 1.62°, 1.08 ± 0.73°, 0.52 ± 0.47° to 0.00 ± 0.30°. With increasing frequency, only the phase angle of β-LG gradually increases from 9.46 ± 0.90° to 19.39 ± 2.04°.

Within the amplitude sweep, β-LG shows a typical viscoelastic behavior with loss in elastic portions with increasing amplitude (**Figure V-6**, page 76). The presence of GS 37 shifts the viscoelastic behavior to a more elastic one (a_2 to c_2) with a phase angle of 0.39 ± 0.71° at 4.2% deformation. The presence of MD 14 and MD 9 results in a shift to a more elastic response (a_3 to c_3 and a_4 to c_4) with a phase angle of -0.06 ± 0.52° and 0.05 ± 0.26° at 4.2% deformation, respectively. Nevertheless, MD 14 and MD 9 show partially a different behavior from amplitudes beyond 4.2%. In one case the compression of the β- LG/MD 14 drop is partially impeded from an amplitude of 5.6%. Changes within the length and orientation of the Lissajous-plot appeared in two cases from an amplitude of 6.3% (c_3). In the presence of MD 9 the compression is once incomplete from an amplitude of 4.9%. Once, the drop collapsed from an amplitude of 5.6%.

For all investigated frequencies and amplitudes, the more elastic response is shown in a linear Lissajous-plot and a phase angle around zero. The linear viscoelastic area ends at 6.3% amplitude with changes in orientation and length of the Lissajous-plots in the presence of MD 14. Baeza et al. (2004) and Perez et al. (2010) showed an increase in elastic response for β-LG with addition of xanthan as well but have not observed the end of the linear viscoelastic area (Baeza et al., 2004; Perez et al., 2010). In comparison, the pure β- LG-film showed a frequency and amplitude dependent behavior in the dilatational rheology (**Figure V-5** and **Figure V-6**), which was shown earlier (Böttcher et al., 2017; Rühs et al., 2013).

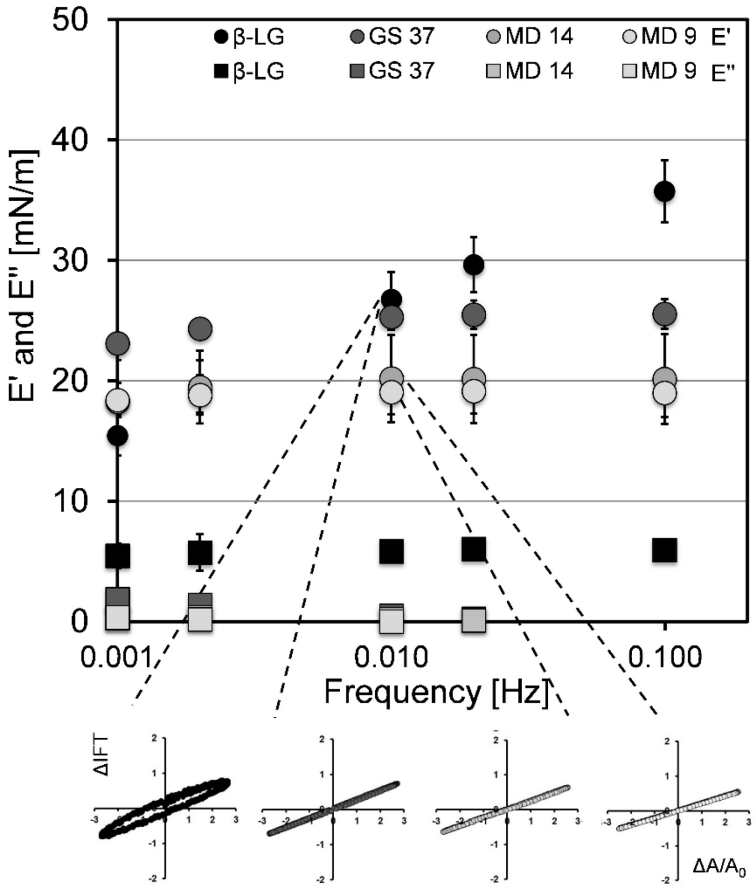

Figure V-5: Frequency sweep with elastic (E') and viscous (E'') moduli for 0.1% β-LG with presence of 34.9% glucose syrup (DE 37) and maltodextrin (DE 14 and DE 9) at MCT-oil/ water-interface, 2.8% amplitude 0.001 – 0.1 Hz after 14 h film formation and Lissajous-plots at 0.01 Hz.

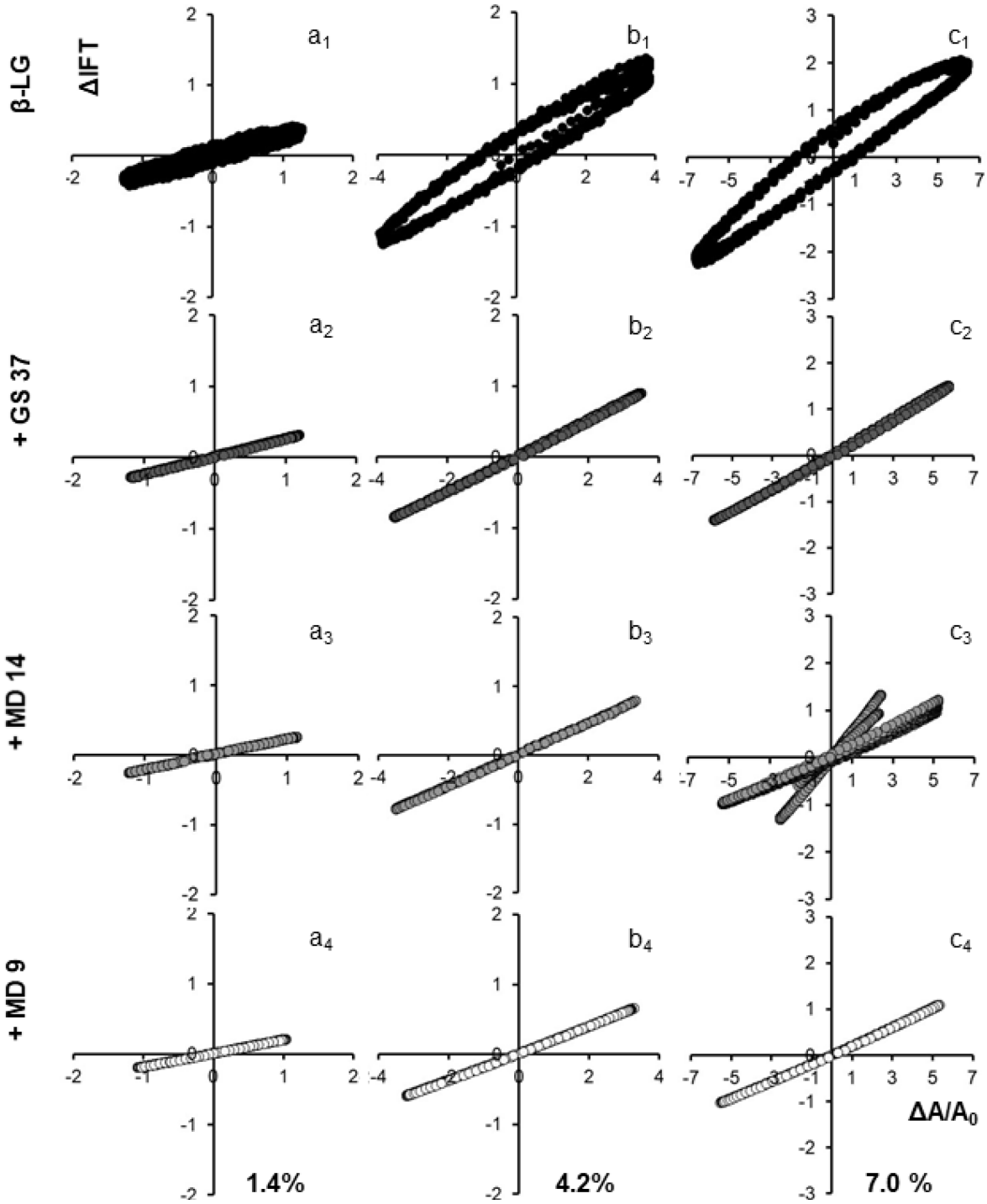

Figure V-6: Representative Lissajous-plots of amplitude sweep for 0.1% β-LG (a_1 to c_1) with presence of 34.9% glucose syrup (DE 37, (a_2 to c_2)) and maltodextrin (DE 14, (a_3 to c_3) and 9, (a_4 to c_4)) at MCT-oil/ water-interface, 1.4%, 4.2 % and 7.0% amplitude and 0.01 Hz after 14 h film formation.

V.3.6 Interfacial shear rheology

The interfacial shear rheology is applied to analyze the viscoelastic interfacial network and its intermolecular interactions. One sample (β-LG and MD 9) showed a low G'. The sample might have been influenced by released water due to partial retrogradation (Shujun Wang et al., 2015). The impact of starch conversion products' presence with varying DE onto the formation of β-LG-films is shown in **Figure V-7** a. At first, the interfacial film is formed and observed for 23 h. The elastic modulus with presence of GS 37 and MD 14 is significantly higher than the elastic modulus of β-LG without presence of starch conversion products and with presence of MD 9 (**Figure V-7** a). The pairwise comparison reveals a significant difference for presence of all starch conversion products in comparison with β-LG in the viscous modulus. The formed interfacial films are further characterized via frequency (**Figure V-7** b) and amplitude sweep (**Figure V-8**, page 78).

Within the frequency sweep, differences in the curve progressions of β-LG with and without presence of starch conversion products are shown (**Figure V-7** b). The elastic modulus is increasing linearly, and the viscous modulus remains constant from 0.01 to 0.25 Hz for all curves. From 0.25 Hz to 1 Hz, the viscous modulus starts to increase while the elastic modulus is reduced. This downturn of the elastic modulus can be explained by instrument inertia which is explained (Radtke et al., 2018).

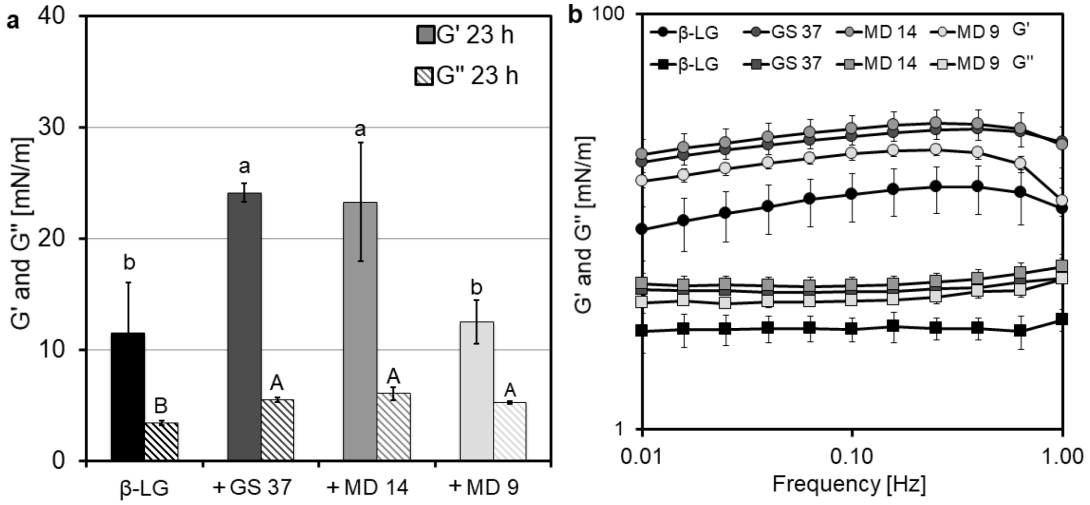

Figure V-7: a) Elastic (G') and viscous modulus (G'') of 23 h aged film and b) frequency sweep with elastic (G') and viscous (G'') moduli for 0.1% β-LG with presence of 34.9% glucose syrup (DE 37) and maltodextrin (DE 14 and 9) at MCT-oil/ water-interface, a) 1 Hz and 0.1% amplitude and b) 0.1% amplitude 0.01 – 1 Hz after 23 h film formation, letters a-b, A-B indicate significant differences ($p < 0.05$).

Within the amplitude sweep, differences in intersection point of G’ and G’’ are present (**Figure V-8**). **Figure V-8** a shows the overall development of the elastic and viscous modulus while **Figure V-8** b pictures the interaction point of G’ and G’’. Significant differences in the intersection point of G’ and G’’ for the y coordinate -G modulus- are shown with presence of MD 14 and GS 37 in comparison to β-LG (**Figure V-8** b). On trend, the G modulus is increased with presence of starch conversion products (**Figure V-8** b). While the x-coordinate in the intersection point -deformation- indicates no significant differences.

The elastic and viscous moduli increase significantly with presence of starch conversion products except for the retrogradation influenced MD 9. Therefore, the different conformational reorganization and intermolecular interactions of stage 3 and 4 result in general in a strong network upon presence of starch conversion products.

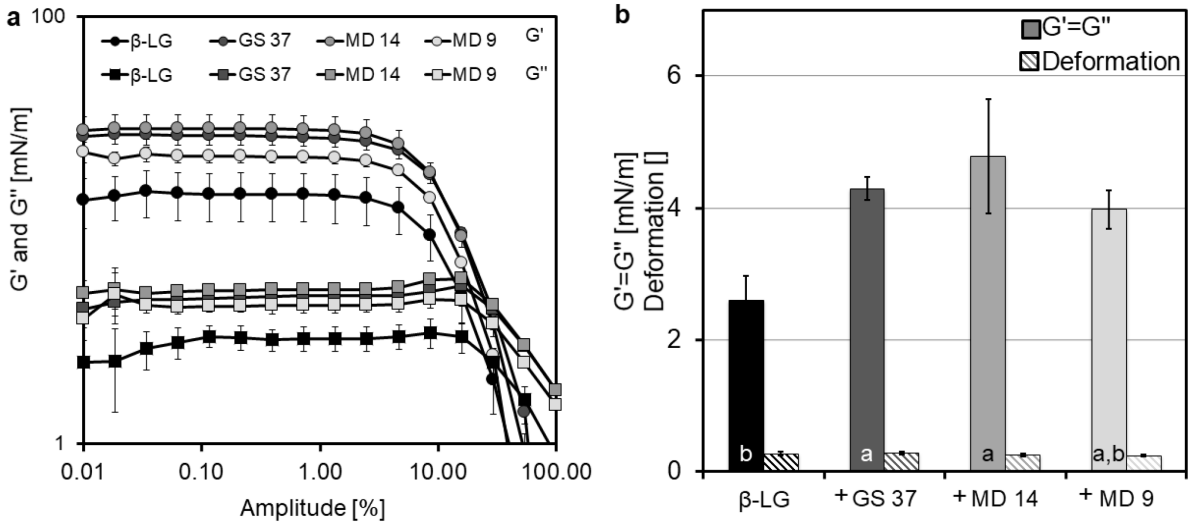

Figure V-8: a) Elastic (G’) and viscous modulus (G’’) of amplitude sweep with b) intersection point for 0.1% β-LG with presence of 34.9% glucose syrup (DE 37) and maltodextrin (DE 14 and 9) at MCT-oil/ water-interface 0.01-100% amplitude at 0.3 Hz, letters indicate significant differences ($p < 0.05$).

V.4 Conclusion

This study focused on the impact of starch conversion products with varying dextrose equivalent at high concentrations on the interfacial properties of β-LG. It was shown that interfacial rheology can be applied at high dry matter content and that a complex interplay between formulation components of emulsions affect the interfacial characteristics. Moreover, interfacial rheology can be used to prove the impact of concentration depended effects like thermodynamic mechanisms.

In general, the presence of starch conversion products supports the formation of a stable interfacial film by the concentration depended excluded volume effect. Our suggestion, that the presence of starch conversion products would influence the interfacial properties of β-LG was confirmed by the increasing elasticity of the interfacial film with increasing intermolecular interactions. A low degree of degradation of starch conversion products resulted in extended protein enrichment at the interface, a high packing density in the film and a lower interfacial tension due to the increased thermodynamic incompatibility.

We assume that for a multicomponent system of oil, starch conversion products, milk proteins and low molecular weight emulsifiers like lecithins, citrem and mono- and diglycerides additional interactions between the oil phase and the low molecular weight emulsifier as well as interactions between the protein and the low molecular weight emulsifier will play a role in interfacial stabilization. Therefore, the impact of interactions between formulation components of spray dried emulsions on the interfacial properties of milk proteins needs to be investigated. The upcoming results will help to understand the mechanisms and interactions involved in the interfacial stabilization of spray dried emulsions which will have an impact on the stability of spray dried powders.

VI. Manuscript 4

Impact of saturation of fatty acids of phosphatidylcholine and oil phase on properties of β-lactoglobulin at the oil/water interface

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Abstract

Oil in water emulsions are commonly stabilized by emulsifying constituents like proteins and/or low molecular weight emulsifiers. The emulsifying constituents can compete or coexist at the interface. Interfacial properties thus depend on molecular structure of the emulsifying constituents and the oil phase and the resulting molecular interactions. The present study systematically analyzed the impact of fatty acid saturation of triacylglycerides and phosphatidylcholine on the interfacial properties of a β-lactoglobulin-stabilized interface. The long-term adsorption behaviour and the viscoelasticity of β-lactoglobulin-films were analyzed with or without addition of phosphatidylcholine via drop tensiometry and dilatational rheology. Results from the present study showed that increasing similarity in fatty acid saturation and thus interaction of phosphatidylcholine and oil phase increased the interfacial tension for the phosphatidylcholine alone or in combination with β-lactoglobulin. The characteristics and stability of interfacial films with β-lactoglobulin-phosphatidylcholine are further affected by interfacial adsorption during changes in interfacial area and crystallization events of low molecular weight emulsifiers. This knowledge gives guidance for improving physical stability of protein-based emulsions in foods and related areas.

Keywords: emulsifier, protein, interactions, rheology, emulsion, crystallization

Graphical abstract

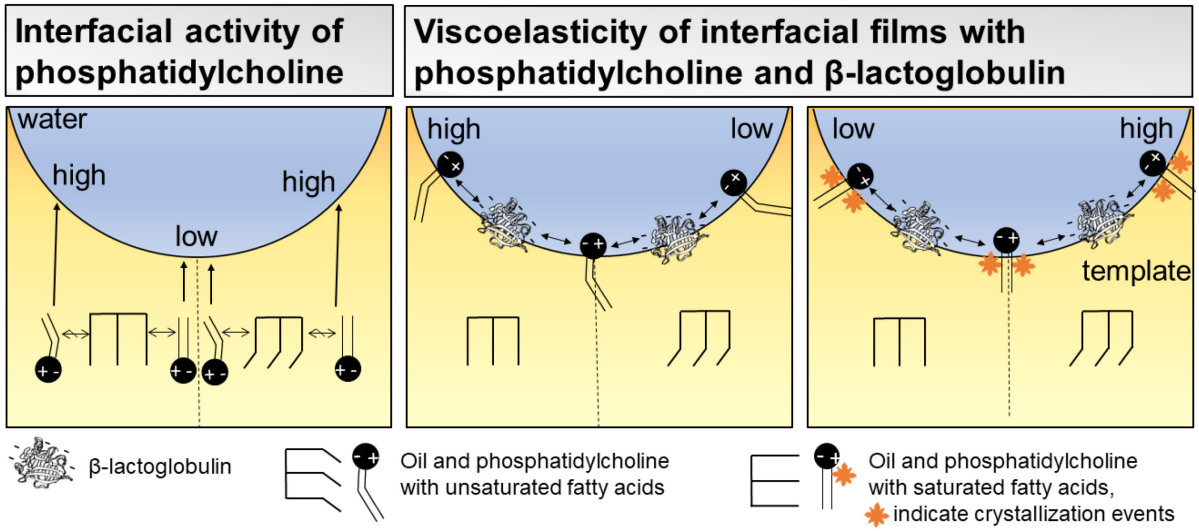

Figure VI-1: Graphical Abstract: Impact of saturation of fatty acids of phosphatidylcholine and oil phase on properties of β-lactoglobulin at the oil/water interface.

VI.1 Introduction

Oil in water emulsions are common systems in the food or pharmaceutical industry. The immiscible phases are stabilized with emulsifying constituents namely proteins and/or low molecular weight emulsifiers. Both types of emulsifying constituents have the ability to decrease the interfacial tension of the system whereby their stabilizing mechanism at the oil/water interface is rather different (Wilde et al., 2004). Proteins are dissolved in the aqueous phase. The molecular structure of the proteins is determined by their amino acid sequence and the folding of the protein (Lam & Nickerson, 2013). They adsorb at the interface, unfold and form a viscoelastic layer with several inter- and intramolecular interactions (Dickinson, 2011; Pugnaloni et al., 2004). An interfacial layer with high viscoelasticity is favourable concerning the stability of emulsions against coalescence (Vega & Roos, 2006; Wilde et al., 2004). In comparison, depending on their molecular structure low molecular weight emulsifiers can be dissolved in the aqueous phase or in the oil phase (McClements & Mahdi Jafari, 2018; Pasquali et al., 2009). Their molecular structure consists of a hydrophilic head group like e.g. phosphatidylcholine and fatty acids with different degree of saturation and chain length (Whitehurst, 2004). They have a high interfacial activity and stabilize emulsions or foams based on the Gibbs-Marangoni mechanism and/or weak electrostatic interactions (Murray & Dickinson, 1996; Wilde et al., 2004).

In a wide range of applications, proteins and low molecular weight emulsifiers are actively used in combination or co-occur through their presence in specific food ingredients. The molecular structure of both emulsifying constituents defines their interactions and interfacial arrangement. In general, proteins can be displaced by highly interfacial active low molecular weight emulsifiers (Bos & van Vliet, 2001; Wilde et al., 2004) or both can coexist at the interface. Coexistence can result in interactions via hydrogen bonding, hydrophobic or electrostatic effects (Dan et al., 2013; Kotsmar et al., 2009; McClements & Mahdi Jafari, 2018). Electrostatic effects depend on the isoelectric point of the proteins (Lam & Nickerson, 2013), the pk_a value for low molecular weight emulsifiers (Cui & Decker, 2016) and the characteristics of the aqueous phase like pH or ionic strength (Lam & Nickerson, 2013). The multitude of interactions between emulsifying constituents determines the characteristics and viscoelasticity of interfacial films and thus emulsion stability. A film with low viscoelasticity is a result of a low number of, repulsive or hydrophobic interactions

(Murray & Dickinson, 1996; Wilde et al., 2004). In contrast, a film with high viscoelasticity results from attractive interactions (Murray & Dickinson, 1996).

The characteristics of a mixed interfacial film with proteins and low molecular weight emulsifiers also depend on the interactions with the lipophilic phase. Variables affecting these interactions are the chemical nature of the lipophilic phase, the structure of the protein as well as the head group and the type of fatty acids in the hydrophobic tail of the low molecular weight emulsifier. Although different lipophilic phase have already been used to study the interfacial properties of dairy proteins (Böttcher et al., 2017; Lucassen-Reynders et al., 2010; Mitropoulos et al., 2014; Wüstneck et al., 1999), no systematic investigation on the impact of interactions between proteins and oil phase on interfacial properties exists. In other recent studies, Bergfreund et al. investigated the impact of alkanes or alkane substituents as lipophilic phases on interfacial tension and viscoelasticity of several protein sources or the interactions between lipophilic phases and surfactants with the same hydrophobic tail but different head group (Bergfreund et al., 2018; Bergfreund, Bertsch, et al., 2021; Bergfreund, Siegenthaler, et al., 2021). To the best of our knowledge, systematic studies on low molecular weight emulsifiers with similar head group, but different fatty acid chains and different oil phases are not available.

In general, interactions between lipophilic components comprise dispersion forces or π-interactions of double bindings (Belitz et al., 2009; Walstra, 2003). The interaction of the low molecular weight emulsifiers and the oil phase are stronger with increasing similarity in fatty acid chain length and saturation as well as solubility of the emulsifier in the lipophilic phase (Garti & Yano, 2001). Since saturated fatty acids have a linear fatty acid chain and unsaturated fatty acids have a kinked chain with an angle of 40° per double bond (Belitz et al., 2009), interactions between unsaturated and saturated fatty acids are not strong and hindered by steric issues. As a consequence, the interfacial occupation and arrangement can be influenced by the interactions between the lipophilic constituents. A recent study from Hildebrandt et al. (2016) showed that strong interactions between lipophilic phase and low molecular weight emulsifier increase the concentration required to reach a monolayer concentration at the interface (Hildebrandt et al., 2016). This study investigated phosphatidylcholine with unsaturated or saturated fatty acids in a lipophilic phase with double bindings (Hildebrandt et al., 2016). Finally, the structure of the low molecular weight emulsifiers and oil phase affect

the crystallization behaviour and morphology of the lipophilic components as well as their interactions. Depending on the phase transition behaviour, low molecular weight emulsifiers may form a template for heterogenous nucleation at the interface (Garti & Yano, 2001). A crystallized low molecular weight emulsifier may be detected with an increase in interfacial viscoelasticity, even if proteins like casein or whey protein are present (Golding & Sein, 2004; Rodríguez Patino, Rodríguez Nino, et al., 2001; Sánchez & Rodríguez Patino, 2004).

Therefore, the aim of the present study is to determine the impact of lipophilic interactions of phosphatidylcholine and oil phase in dependence on saturation of fatty acid chains on the interfacial properties of mixed interfacial films. It is hypothesized that the interfacial tension is higher for a phosphatidylcholine with increasing interactions with the oil phase. At low concentrations, the interfacial tension of a β-lactoglobulin-phosphatidylcholine stabilized interface is decreased due to coexistence of the emulsifying constituents at the interface. An interfacial film stabilized with β-lactoglobulin-phosphatidylcholine reacts more elastic during expansion and compression if the phosphatidylcholines are not strongly interacting with the oil phase. This effect is caused by a higher concentration of phosphatidylcholines at the interface, faster stabilization of unoccupied interfacial regions or a solid template at the interface depending on saturation of fatty acids of phosphatidylcholines.

To fulfil the aim, we investigated two phosphatidylcholine samples differing in the fatty acid composition, but similar in their head group and two oils differing in fatty acid composition. The development of interfacial tension was measured with or without addition of phosphatidylcholines via drop tensiometry in a long-term time range. The viscoelasticity of the corresponding interfacial films was investigated using dilatational rheology.

VI.2 Materials and methods

β-lactoglobulin was isolated from whey protein isolate (Bipro, Agropur Dairy Cooperative Inc. Minnesota, USA) with a method described elsewhere (Keppler et al., 2014; Schestkowa et al., 2019). The resulting protein powder has a dry matter content of 92.8 ± 1.2% and a protein content of 92.8 ± 0.4%. The protein fraction consists of 99.6% isolated β-lactoglobulin and 0.4% α-Lactalbumin which was analyzed according to (Keppler et al., 2014). Medium chain triglyceride oil (MCT-oil) WITARIX® MCT 60/40

was kindly provided from IOI Oleo GmbH (Hamburg, Germany). Sunflower seed oil from *Helianthus annuus* was purchased from Sigma Aldrich Chemie GmbH (Steinheim, Germany). The MCT-oil consists of 0.1% C6:0, 56.4% C8:0, 43.3% C10:0 and 0.1% C12:0 and has an acid value of 0.04%. The sunflower oil consists of 6% C16:0, 4% C18:0, 26% C18:1 and 63% C18:2 and has an acid value of 0.09%. Both oils were treated with magnesium silicate (Florisil® from Carl Roth GmbH, Karlsruhe, Germany) to remove interfacial active substances. The interfacial tension was controlled to be constant via drop tensiometry. After purification, the sunflower oil was immediately frozen in portions and was thawed for each experiment individually. Two phosphatidylcholines were kindly provided from Lipoid GmbH (Ludwigshafen, Germany). Phospholipon®90 H consists of molecules with 96.9% phosphatidylcholine as head group with fatty acids of 98% of C16:0 and C18:0, 0.1% C18:1 and C18:2. Lipoid S100 consists of 97.4% phosphatidylcholine as head group with fatty acids of 12-17% C16:0, 2-5% C18:0, 7-12% C18:1, 59-70% C18:2 and 5-8% C18:3. Both phosphatidylcholines are obtained from soy origin.

VI.2.1 Preparation of protein solutions

For adsorption behaviour and dilatational rheology measurement, β-lactoglobulin was dissolved and stirred in distilled water for about 2 h. The pH of the 0.01 d.m.% β-lactoglobulin solution was adjusted to pH 7 with 0.1M and 1M NaOH. The solutions were stored at 5 °C for about 14 h overnight. Afterwards, the solutions were stirred, and the pH was adjusted if necessary.

VI.2.2 Preparation of phosphatidylcholine-oil solutions

The phosphatidylcholines were dissolved in MCT-oil or sunflower oil at a concentration of 0.1%. These oil-phosphatidylcholine solutions were used as stock solution and were heated to the melting of the respective phosphatidylcholines. The choice of the heat treatment was made according to the specification sheet and visual observation as shown in **Figure VI-2** at page 88. 90H-solutions were heated to 90 °C for 15 min and S100 solutions were heated to 50 °C for 15 min. The heat-treated stock solutions were diluted to investigate the adsorption behaviour of phosphatidylcholines alone or in combination with β-lactoglobulin.

VI.2.3 Long term adsorption behaviour of phosphatidylcholines with or without β-lactoglobulin

Long term adsorption behaviour was measured by drop tensiometry (PAT1M, Sinterface Technologies e.K., Berlin, Germany). The drop shape is recorded with a high-speed camera and the interfacial tension is calculated from the drop curvature with the help of the improved Young Laplace equation. The droplet size was chosen at 30 mm³.

Concentration series of phosphatidylcholines were recorded from 0.1%, 0.01%, 0.001%, 0.0005% to 0.0001%. The diluted phosphatidylcholine-oil solutions were heated to 90 °C for 8 min before measurement to ensure melting of phosphatidyl-cholines. A droplet of distilled water was formed in MCT-oil-phosphatidylcholine solutions at 60.5 ± 1.5 °C. The interfacial tension was recorded for 30 min including the 10 min cooling period to room temperature. In **Figure VI-2** at page 88, representative MCT-oil-phosphatidylcholine solutions are shown. For all concentrations, the measurement with visual equipment like a drop tensiometer is feasible. Only the investigation of the stock solution with 0.1% 90H is not feasible, if the temperature approaches a value of 50 °C or below. This solution developed a high turbidity caused by crystallization.

The characteristics of interfacial films with phosphatidylcholines and β-lactoglobulin were measured with one chosen phosphatidylcholine concentration of 0.0001%. Therefore, a 0.01% β-lactoglobulin droplet was formed in a MCT-oil or sunflower-oil-phosphatidylcholine mixture. The phosphatidylcholine-oil heat and cooling treatment was the same as described above whereby the interfacial tension was recorded for 3 h. For long term adsorption behaviour, a method standard deviation was calculated based on five replicated measurements of a representative β-lactoglobulin-phosphatidylcholine sample.

VI.2.4 Dilatational rheology

Dilatational rheology was investigated by pendant drop tensiometry (PAT1M, Sinterface Technologies e.K., Berlin, Germany). A high-speed camera was used to record the change in drop shape during sinusoidal oscillation.

A β-lactoglobulin droplet was formed in MCT-oil or sunflower-oil-phosphatidylcholine mixtures. The drop was equilibrated for 3 h (see long term adsorption behaviour).

Subsequently, a frequency sweep (2% amplitude, 0.002 Hz to 0.1 Hz) followed by an amplitude sweep (0.01 Hz, 1% to 5% amplitude) were performed.

The complex dilatational modulus (E*) is calculated from the proportion of the change in interfacial tension (σ) and area (A) (equation 9) (Lucassen-Reynders, 1993). The elastic modulus (E') and the viscous modulus (E'') are determined with the help of equation 10. The phase angle (ϕ) is calculated with tan(ϕ) = E''/E' (Lucassen-Reynders, 1993). A phase angle of 0° represents entirely elastic behaviour and a phase angle of 90° represents entirely viscous behaviour. A value between 0° and 90° is attributed to viscoelastic behaviour of the film.

$$E^* = \frac{d\sigma}{d \ln A} \quad (9)$$

$$E^* = E_d + i\omega\eta_d = E' + iE'' \quad (10)$$

Beside the described dilatational moduli and phase angle, the data of dilatational rheology is presented with Lissajous-plots. These figures show the change in interfacial tension (ΔIFT = σ-σ_0) versus the change in area ($\Delta A/A_0$; $\Delta A = A - A_0$). σ_0 and A_0 represent the interfacial tension and area at zero strain. For dilatational rheology, a method standard deviation was calculated based on five replicated measurements of a representative sample.

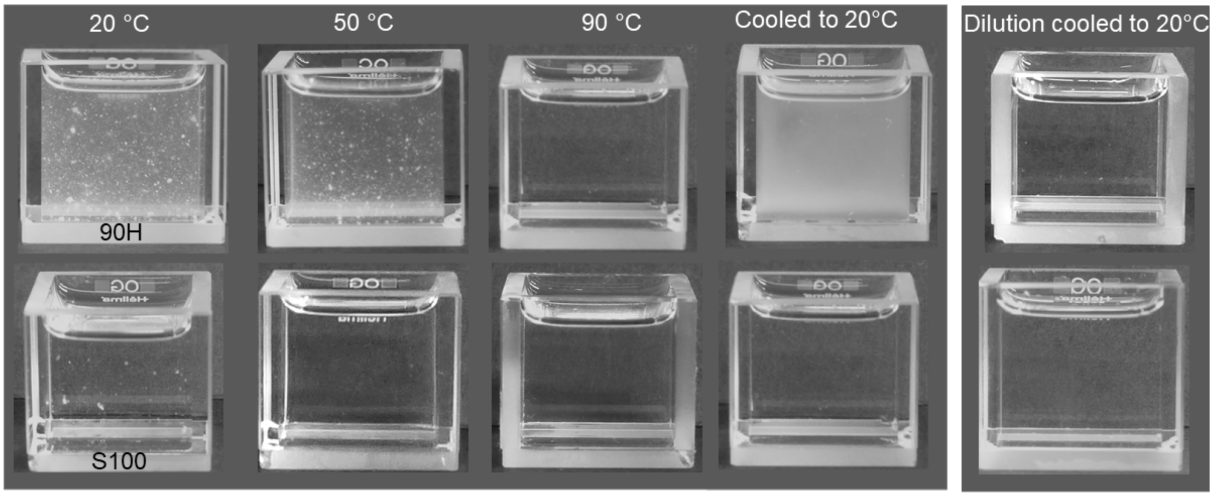

Figure VI-2: Images of solutions with 0.1% phosphatidylcholines (S100 or 90H) in MCT-oil during heating to 90 °C and subsequent cooling to 20 °C. Diluted phosphatidylcholine-MCT-oil solutions with a concentration of 0.0001% cooled to 20 °C.

VI.2.5 Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA). Significance was measured by post-hoc Scheffé test ($p < 0.05$) using IBM SPSS statistics version 28.0.0.0 (IBM Corp., Armonk, USA). Statistical results are displayed in Fig.3 and 4, and in Fig.5 and 7 for interfacial tension and elastic moduli, respectively. One may pay attention when discussing the outcome of the statistical analysis. It is based on the typical standard deviation of the methodology as determined from multiple measurements of a representative sample from these experiments, but not individual independent measurements of each sample.

VI.3 Results and Discussion

VI.3.1 Long term adsorption behaviour of phosphatidylcholines in MCT-oil

The interfacial tension of the pure oil-water interface amounted to 25.4 ± 0.5 mN/m. High concentrations of 0.1% and 0.01% of the low molecular weight emulsifiers were not analyzed, since the droplets detached from the needle during the measurement. During long term adsorption in the concentration range of 0.001% to 0.0001%, phosphatidylcholines in MCT-oil decreased the interfacial tension depending on their fatty acid composition (**Figure VI-3**, page 91). The low molecular weight emulsifier with predominantly unsaturated fatty acids, S100, shows a higher interfacial activity in comparison to 90H with saturated fatty acids in the hydrophobic tail (**Figure VI-3**). The interfacial tension of S100 decreased after 30 min to 24.5 ± 0.4 mN/m, 18.0 ± 0.3 mN/m to 3.9 ± 0.1 mN/m with increasing concentration (**Figure VI-3** a). For 90H, the interfacial tension was reduced to 23.8 ± 0.4 mN/m, 21.1 ± 0.3 mN/m to 20.6 ± 0.3 mN/m with increasing concentration (**Figure VI-3** b).

These differences in interfacial tension can be attributed to the interactions and solubility of the low molecular weight emulsifiers in the oil as well as the crystallization behaviour. In general, the interactions of low molecular weight emulsifiers and oil phase as well as solubility of the low molecular weight emulsifier in the oil phase increase with increasing similarity in chain length and saturation (Garti & Yano, 2001). A low molecular weight emulsifier with unsaturated and kinked fatty acids is not strongly integrated in an oil phase with linear nature of saturated fatty acids (Belitz et al., 2009). Thus, the phosphatidylcholine S100 moves faster and more easily to the interface. In contrast, 90H contains mainly fatty acids of C16:0 and C18:0 which can interact strongly with saturated fatty acids C10:0 and C8:0 of the MCT-oil. The saturated fatty acids of phosphatidylcholines gets more easily integrated in the tightly packed oil phase with saturated and linear fatty acids (Belitz et al., 2009) and interacts via dispersion forces (Walstra, 2003).

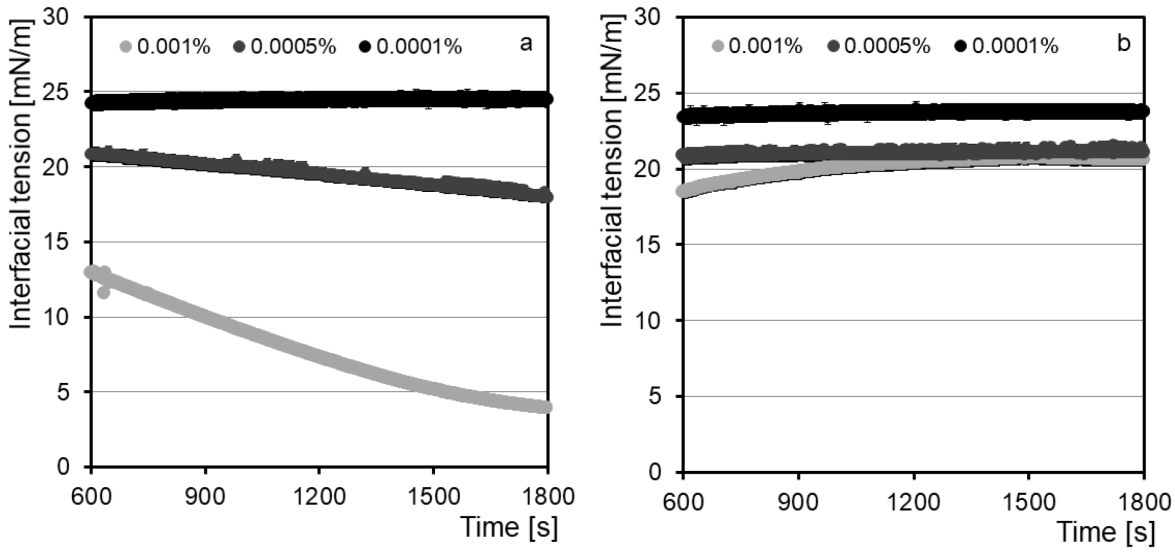

Figure VI-3: Interfacial tension of concentration series of phosphatidylcholines S100 (a) and 90H (b) in MCT-oil in a concentration range from 0.001 to 0.0001% at room temperature. Error bars display the coefficient of variation of the method.

Furthermore, the phosphatidylcholine 90H crystallizes during the cooling period (**Figure VI-2**, page 88). If crystallization occurs at the interface and the phosphatidylcholine is covering a high portion of the interface, a network of phosphatidylcholine crystals may induce a change in the drop shape. This was observed as an increase in interfacial tension for the sample 0.001% 90H at 600 s to 900 s. In previous studies, a rather severe drop shape transformation was observed alongside the use of emulsifiers with long saturated fatty acids during liquid-solid phase transition at the oil/water interface of emulsions (Denkov et al., 2015, 2019). It was described that the drop can be deformed by multilayers of emulsifiers at the interface which are inducing a high bending moment which curves the drop shape against the drop surface energy (Denkov et al., 2015, 2019). Therefore, the forces of liquid-solid phase transition at interfaces may deform the droplet and affect the analysis of interfacial tension. This effect is measurable from a certain concentration covering a high portion of the droplet interface.

In summary, higher interactions of phosphatidylcholines with the oil phase leads to a lower reduction of the interfacial tension, since occupation of the interface and thus amount of phosphatidylcholine molecules and packing density at the interface are reduced. For long term adsorption of β-lactoglobulin and phosphatidylcholines, and dilatational experiments, we have chosen the lowest phosphatidylcholine concentration of 0.0001%.

VI.3.2 Long term adsorption of β-lactoglobulin and phosphatidylcholines in MCT-oil

Interfacial tension measurement on a long-term may be used to verify coexistence of the emulsifying constituents. Thus, the interfacial tension of β-lactoglobulin with or without presence of the phospholipids 90H and S100 was analyzed after 3 h droplet ripening time (**Figure VI-4**, page 93). β-lactoglobulin at 0.01% lowered the interfacial tension of water against MCT-oil from 25.4 ± 0.5 mN/m to 20.5 ± 0.4 mN/m. This value is approx. 2.5 mN/m higher than reported previously for 0.01% β-lactoglobulin-film after a droplet ripening time of 1 hour (Schestkowa et al., 2020). On one hand this may attributed to differences in time of analysis and inherent properties of the β-lactoglobulin (e.g., genetic variants, degree of aggregation). On the other hand, the short exposure to elevated temperature immediately after drop generation might have affected the interfacial organization and occupation. It is known, that heat exposure of β-lactoglobulin located at the interface barely changes the molecular structure of the protein (Zhai et al., 2010), but dynamics of adsorption and interfacial arrangement might be affected by a thermally induced change in mobility.

In both cases 0.0001% S100 or 90H, a combination of β-lactoglobulin and phosphatidylcholine led to a more pronounced and significant reduction in interfacial tension at the MCT oil-water interface compared to the protein or low molecular weight emulsifiers alone. The interfacial tension decreased to 16.9 ± 0.3 mN/m in the β-lactoglobulin-S100 system and to 18.5 ± 0.4 mN/m in the β-lactoglobulin-90H system. Therefore in both cases, coexistence of the emulsifying constituents can be assumed. This assumption is further confirmed by data on the critical interfacial concentration. For β-lactoglobulin full interfacial coverage was stated to be slightly above 0.1% (Schestkowa et al., 2020), the critical micelle concentration (CMC) with full interfacial coverage ranged between 0.014 to 0.017% for several commercial soy-based phosphatidylcholines solubilized in MCT-oil (unpublished data). Therefore, we assume that the concentration of each individual substance was well below the critical concentration for full coverage. The higher and significant reduction of the interfacial tension in the binary system containing S100 compared to 90H can be attributed to the mechanisms as outlined for the phospholipids above.

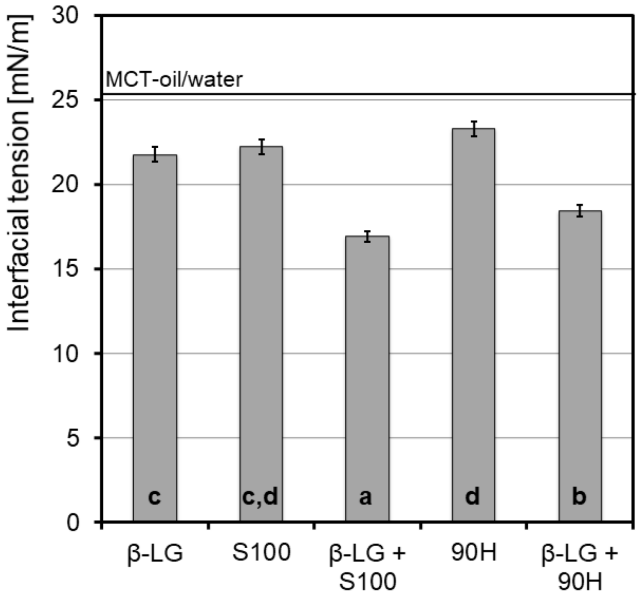

Figure VI-4: Interfacial tension of 0.01% β-lactoglobulin, 0.0001% phosphatidylcholines 90H and S100 in MCT-oil alone or in combination after 3 h droplet ripening time. Error bars display the coefficient of variation of the method, letters a-d indicate significant differences ($p < 0.05$).

VI.3.3 Long term adsorption of β-lactoglobulin and phosphatidylcholines in sunflower-oil

At a sunflower-oil/water interface, β-lactoglobulin lowered the interfacial tension from 29.9 ± 0.5 mN/m to 24.2 ± 0.5 mN/m (**Figure VI-5**, page 94). Thus, the decrease in interfacial tension is very similar to the MCT-oil/water interface after 3 h droplet ripening time (**Figure VI-4**). An impact of oil phase polarity on reduction in interfacial tension of β-lactoglobulin was described (Bergfreund, Bertsch, et al., 2021) and the same authors showed that more polar lipophilic phases interact with hydrophilic moieties of the β-lactoglobulin (Bergfreund et al., 2018). However, in the present study the difference in molecular structure and polarity between MCT-oil and sunflower-oil might be to small to cause a difference in interfacial behaviour for β-lactoglobulin.

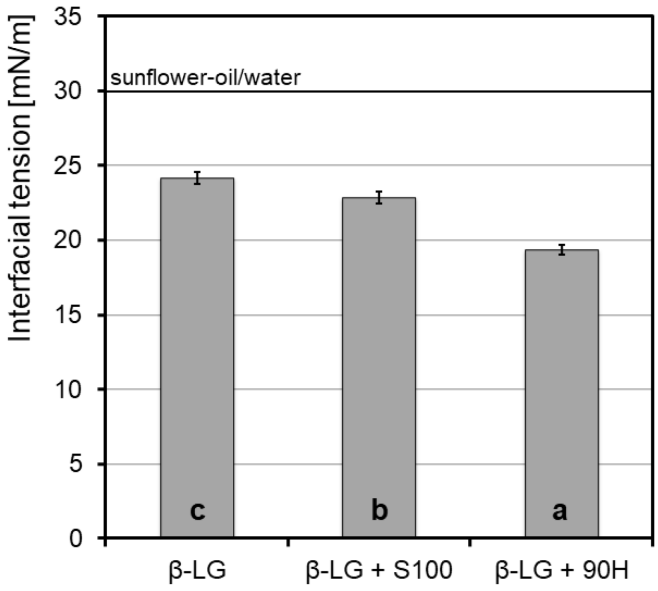

Figure VI-5: Interfacial tension of 0.01% β-lactoglobulin alone or in combination with 0.0001% phosphatidylcholines 90H and S100 in sunflower-oil after 3 h droplet ripening time. Error bars display the coefficient of variation of the method, letters a-c indicate significant differences ($p < 0.05$).

As hypothesized, addition of the saturated phosphatidylcholine 90H lowers the interfacial tension significantly and more strongly (19.3 ± 0.4 mN/m) at the sunflower oil -water interface than the unsaturated phosphatidylcholine S100 (22.8 ± 0.5 mN/m). The unsaturated phosphatidylcholine S100 interacts with the unsaturated oil (Garti & Yano, 2001), which shows a less tightly packed arrangement of fatty acid chains than the saturated oil. The interactions of unsaturated fatty acids of oil phase and low molecular weight emulsifiers are based on very weak π-interactions of the double bondings or dipersion forces (Belitz et al., 2009; Walstra, 2003) and hinder reduction of the interfacial tension. In addition, Hildebrandt et al. (2016) suggested a liquid expanded interfacial layer for an unsaturated phosphatidylcholine in an unsaturated oil and a highly condensed interfacial layer for a saturated phosphatidylcholine in an unsaturated oil (Hildebrandt et al., 2016). A liquid expanded layer gives more space for each emulsifying constiutent than a condensed layer (Pichot et al., 2013). Therefore, we assume a higher amount of phosphatidylcholines 90H fitting on the interface which might reduce the interfacial tension to a higher degree than S100.

VI.3.4 Dilatational rheology of β-lactoglobulin and phosphatidylcholines in MCT-oil

During the frequency and amplitude sweep, the elastic modulus of β-lactoglobulin stays rather constant at approximately 14 mN/m (**Figure VI-6**, page 96). From the amplitude sweep it becomes obvious that the system was in the linear viscoelastic regime and also within the frequency range analyzed no irreversible structural changes in the protein film occurred (**Figure VI-6**). The ratio of the elastic to the viscous modulus is reflected in the phase angle. At an amplitude of 4% the phase angle amounted to 3.3 ± 0.9° for β-lactoglobulin and thus reflects a viscoelastic behaviour with a high elastic contribution. Upon addition of both phosphatidylcholines, for both sweeps the elastic modulus was higher than for the pure protein film (**Figure VI-6**). E' amounted to approximately 24 mN/m in the amplitude sweep and 21 m/N/m in the frequency sweep. Values for an interface stabilized through β-lactoglobulin and 90H were lower with approximately 19 and 18 mN/m, respectively. The viscous modulus (E'') shows no differences between the samples (**Figure VI-6**). The phase angle was lowest in the system with addition of S100 (1.8 ± 0.5°) similar to β-lactoglubulin in the system with addition of 90H (3.0 ± 0.8°).

At first the higher values of E' in the presence of low molecular weight emulsifiers can be ascribed to the coexistence of the two emulsifying constituents. During compression and expansion, desorption and adsorption of interfacial active molecules to and from the interface occurs (Murray & Dickinson, 1996; Wilde et al., 2004). During the increase in area within dilatational rheology, due to the non-ideal shape of the drop surface a gradient in interfacial occupation and thus interfacial tension at the interface causes a force to restore the interface with low molecular weight emulsifiers (similar to the Gibbs-Marangoni flow) (Murray & Dickinson, 1996). Lower values of E' for 90H compared to S100 (in particular shown in **FigureVI-6** b) reflect the stronger interactions between the molecule and the oil phase. The high interactions of the lipophilic components decelerate the de- and adsorption processes upon compression/expansion. A crystalline state of the emulsifier was reported to increase the elastic response in comparison to the protein (Golding & Sein, 2004; Rodríguez Patino, Rodríguez Nino, et al., 2001; Sánchez & Rodríguez Patino, 2004) based on the rigid reaction of the interface. In the study of Patino et al. (Rodríguez Patino, Rodríguez Nino, et al., 2001) monolaurin increased the elastic modulus to a higher degree than monoolein based

on crystallization effects at the interface. However, in the binary system in the present study mobility effects as outlined before obviously dominated the overall behaviour of the system.

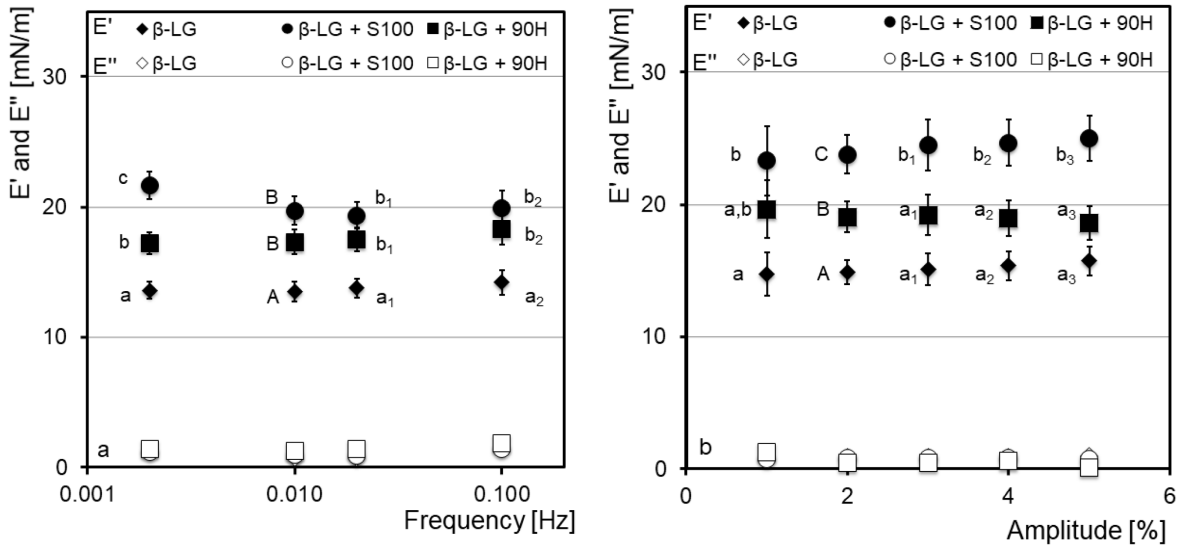

Figure VI-6: Elastic (E') and viscous modulus (E'') of frequency (a) and amplitude sweep (b) of 0.01% β-lactoglobulin alone or in combination with 0.0001% phosphatidylcholines 90H and S100 in MCT-oil. A) amplitude 2%, b) frequency 0.01 Hz. Error bars display the coefficient of variation of the method, letters a-c indicate significant differences between E' for each frequency (a) or amplitude (b) ($p < 0.05$).

The results of the amplitude sweep are depicted as Lissajous-plots (**Figure VI-7**, page 97) to gain more information of the interfacial reaction onto expansion and compression. The β-lactoglobulin-film shows linear and comparably symmetric Lissajous-plots without widening with increasing amplitude. So, the elastic and viscous portions of the film are mostly constant during the amplitude sweep without difference during expansion and compression of the droplet area (Sagis & Fischer, 2014). The addition of S100 shifts the Lissajous-plot to a slightly steeper angle (**Figure VI-7** b_2 and c_2).This shift may be attributed to an increase of the change in interfacial tension during oscillation, which may be caused by a reduced number of interactions stabilizing the protein film at the interface. Adsorption of low molecular weight emulsifiers from the bulk did not fully compensate this effect. For 90H, the interfacial film loses elastic portions with increase in oscillation amplitudes which can be seen in the shift to an ellipsoidal shape of Lissajous-plots with increasing amplitude (**Figure VI-7** b_3 and c_3). This loss is in accordance to the slight loss in the elastic moduli (**Figure VI-6** b) and is attributed to the rigid and crystallized structure at the interface, which tends to break

during expansion and compression (Rodríguez Patino, Rodríguez Nino, et al., 2001). The shown data did not indicate non-linearity as discussed in (Sagis & Fischer, 2014).

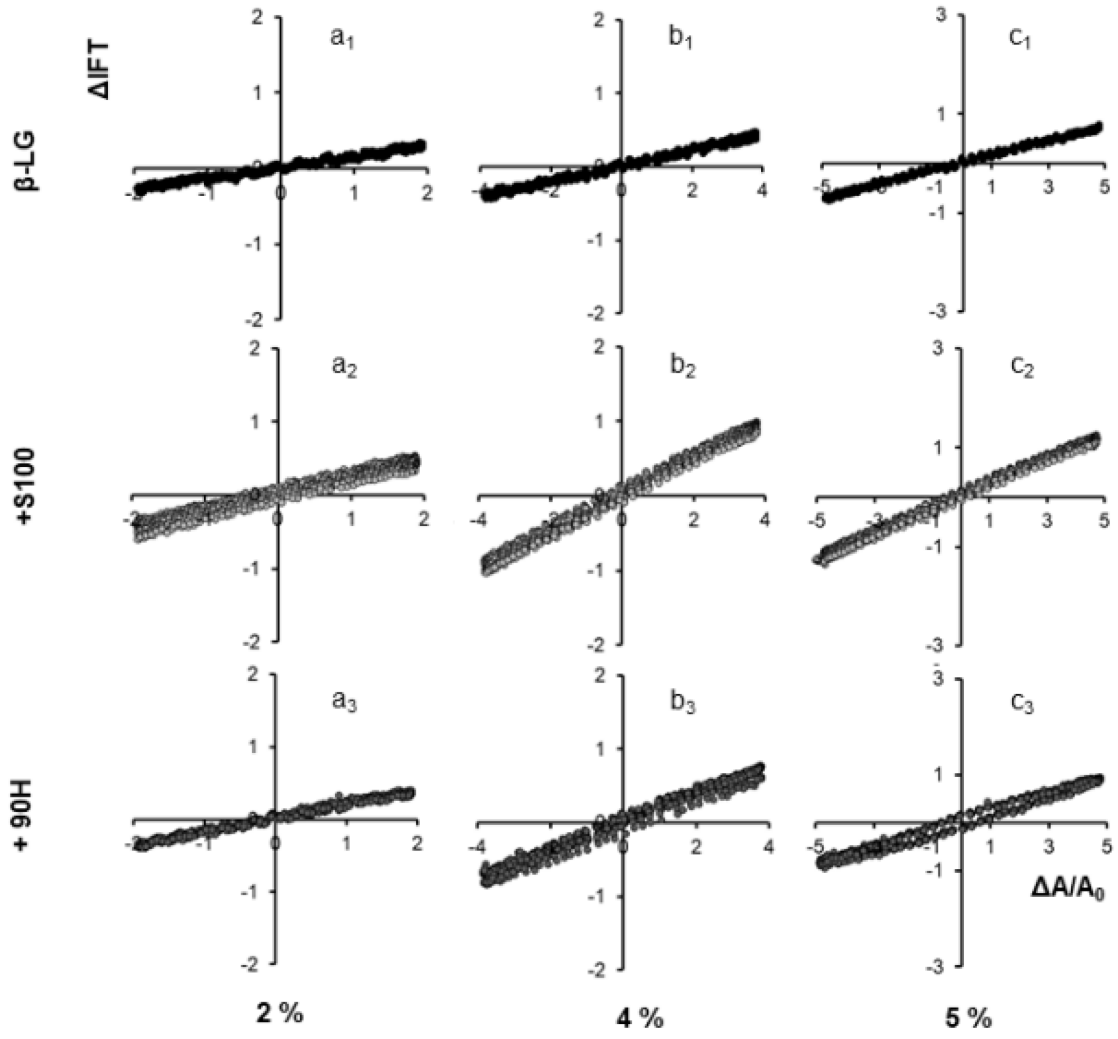

Figure VI-7: Lissajous-plots of amplitude sweep of 0.01% β-lactoglobulin alone (a_1-c_1) or in combination with 0.0001% phosphatidylcholines S100 (a_2-c_2) or 90H (a_3-c_3) in MCT-oil, frequency at 0.01 Hz.

VI.3.5 Dilatational rheology of β-lactoglobulin and phosphatidylcholines in sunflower-oil

The interfacial characteristics of the β-lactoglobulin-film in a sunflower-oil/water interface are comparable to the MCT-oil/water interface (**Figure VI-8**, page 98). During the amplitude and frequency sweep, the elastic moduli of β-lactoglobulin stay rather constant (**Figure VI-8**). The addition of phosphatidylcholines increases the elastic moduli, however, the increase is opposite to the data presented for an MCT-oil-water interface in **Figure VI-6**. For S100, the values are increasing with increasing frequency.

In the presence of 90H, the values for E' are significantly highest and stay almost constant (**Figure VI-8**). The viscous moduli (E'') of the samples are rather similar.

Comparing the data for the two different interfaces, the impact of polarity of the oil phase on the visoelastic behaviour of β-lactoglobulin (Bergfreund et al., 2018) was not shown in our study with triaclyglyceride oils with different degree in saturation. As in the case of the adsorption in **chapter VI 3.2** and **VI 3.3**, the difference in polarity between MCT-oil and sunflower-oil was too small to cause a difference in interfacial behaviour. The mechanisms responsible for the increase in the elastic moduli in the presence of S100 and 90H confirm our previous discussion on the role of interactions with the oil phase and solubility of the low molecular weight emulsifier. The high solubility of S100 results from higher interactions with the oil phase and a reduced presence of S100 at the interface. Therefore in this setup the elastic modulus is significantly highest for 90H (**Figure VI-8**) as a consequence of its comparably lower interactions with the oil phase.

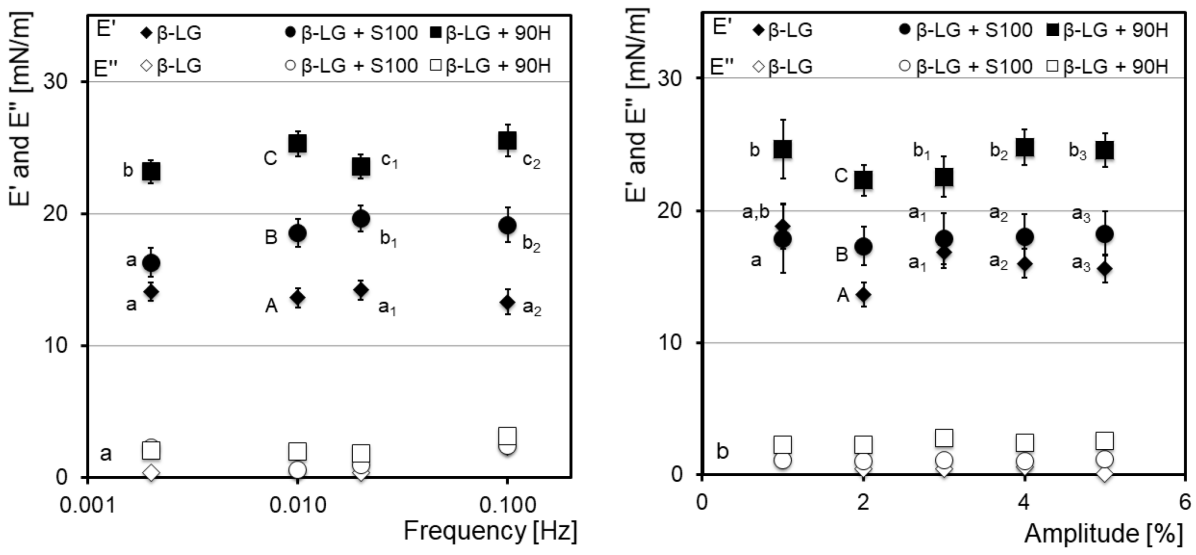

Figure VI-8: Elastic (E') and viscous modulus (E'') of frequency (a) and amplitude sweep (b) of 0.01% β-lactoglobulin alone or in combination with 0.0001% phosphatidylcholines 90H and S100 in sunflower-oil. A) amplitude 2%, b) frequency 0.01 Hz. Error bars display the coefficient of variation of the method, letters a-c indicate significant differences between E' for each frequency (a) or amplitude (b) ($p < 0.05$).

The Lissajous-plots show that the β-lactoglobulin-film remains elastic without apparent loss in elastic portion with increasing amplitude (**Figure VI-9**, page 99). For the addition of S100, Lissajous-plot of the β-lactoglobulin-film with addition of S100 barely show asymmetric tendencies and is mostly symmetric and linear (**Figure VI-9** a_2-c_2). The Lissajous-plot of the sample with 90H shows a steeper angle and is widening with

increasing amplitude showing a loss in elastic portion. The loss in elastic portion might be attributed to a rigid and crystallized structure at the interface (Rodríguez Patino, Rodríguez Nino, et al., 2001). That is in accordance with the increase in phase angle for addition with 90H. A similiar effect of loss in elastic portion was observed in the MCT-oil/water interface (**Figure VI-7**, page 97). In **Figure VI-9**, these effects are even more pronounced since a template with a high phosphatidylcholine concentration at the interface is formed. Crystals at the interface could be observed with the naked eye in the drop image in the software after measurement.

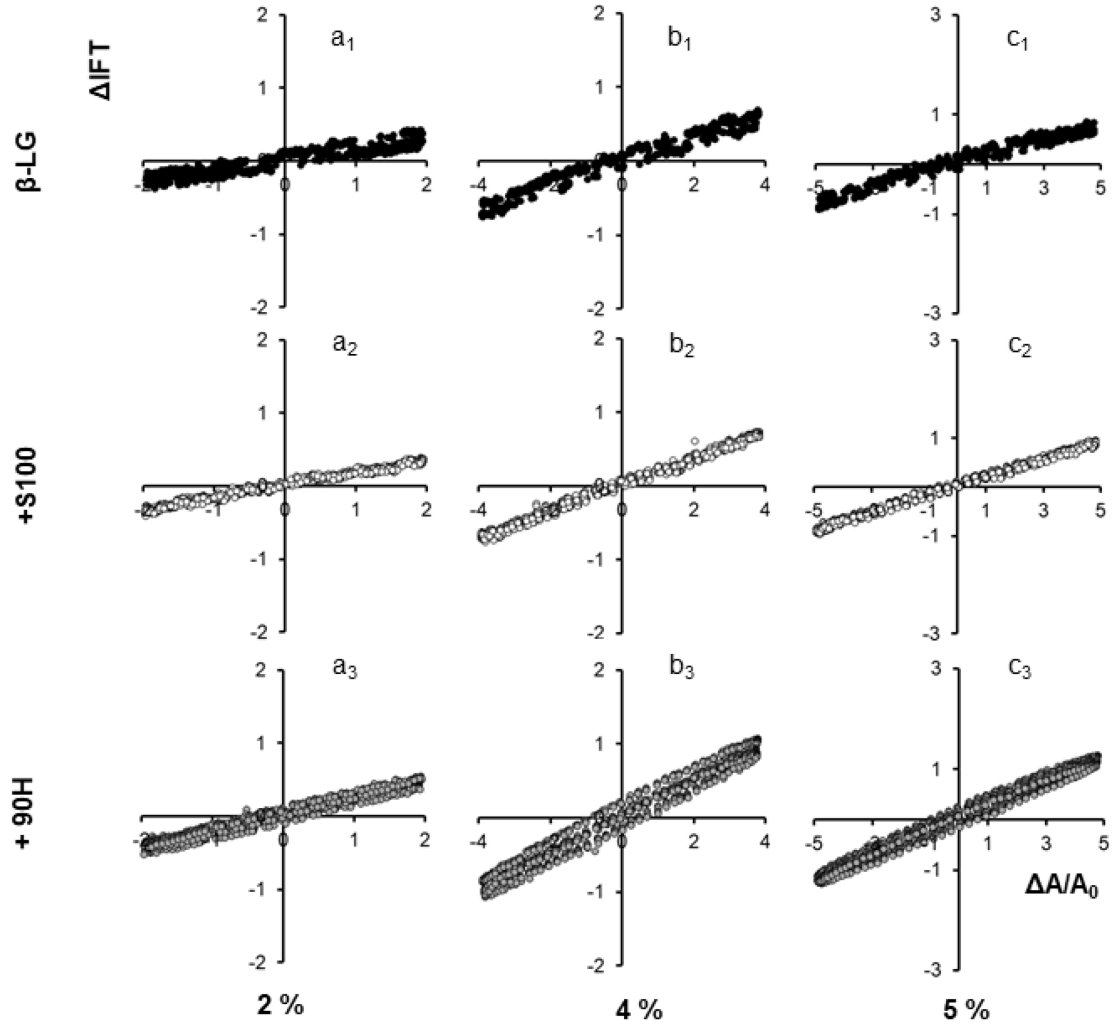

Figure VI-9: Lissajous-plots of amplitude sweep of 0.01% β-lactoglobulin alone (a_1-c_1) or in combination with 0.0001% phosphatidylcholines S100 (a_2-c_2) and 90H (a_3-c_3) in sunflower-oil, frequency at 0.01 Hz.

VI.4 Conclusion

The present study analyzed the impact of interactions and solubility of oil phase and low molecular weight emulsifiers depending on saturation of fatty acids on the interfacial properties of β-lactoglobulin as model protein. The saturation of the oil phase did not affect the interfacial tension or viscoelasticity of β-lactoglobulin-films. In contrast, the saturation of the oil phase had a large impact on interfacial characteristics of phosphatidylcholines. If a phosphatidylcholine is highly soluble in the oil phase, the interactions with the oil phase are reducing its potential to lower the interfacial tension in comparison to a phosphatidylcholine with low solubility. The interactions result in a lower interfacial occupation and higher interfacial tension. In a system with β-lactoglobulin, the interfacial tension is higher if high interactions of phosphatidylcholine and oil phase exist. The characteristics and stability of the interfacial film of β-lactoglobulin-phosphatidylcholine are further affected by interfacial adsorption during changes in interfacial area, and crystallization of low molecular weight emulsifiers. Future research should also cover interfacial rheology in the non-linear regime to get a deeper inside into stress-response of the mixed films.

However, it is already obvious that all these effects are of importance for the application in food or pharmaceutical products. Therefore, the choice of saturation of fatty acids of oil phase and low molecular weight emulsifier is rather important. For instance, a solid template at the interface could hinder oil droplet destabilization by oil crystallization if the emulsion is exposed to temperatures below the oil crystallization temperature during storage time (Fredrick et al., 2013; Goibier et al., 2017). The transferability to other low molecular weight emulsifiers like mono- and diglycerides or citrem or oil phases like alkanes needs to be analyzed. The upcoming results will help to systematically understand the impact of interactions of low molecular weight emulsifiers and oil phase on interfacial properties of proteins.

VII. General discussion

VII.1 Impact of interactions of emulsifying constituents on their performance during processing of spray dried emulsions

This chapter focuses on interactions between emulsifying constituents to explain changes in their performance during the processing steps: emulsification, atomization and drying step.

To elucidate the performance of emulsifying constituents during processing steps, the results of interfacial, emulsion and spray dried emulsion studies before powder formation are connected. Therefore, changes in oil droplet size during processing steps - emulsification, atomization and especially drying step - are linked to the interfacial behaviour of the same emulsifying constituent combination. The performance of emulsifying constituents affected the characteristics of spray dried emulsions. Thus, the oil droplet size of the emulsions changed during emulsification, atomization and drying step (**Chapter III and IV** from page 18 and 39). The investigated emulsifying constituents are whey protein (modelled partly with β-lactoglobulin) with addition of lecithin, citrem or mono-/diglyceride. Maltodextrin and middle chain triglyceride oil were used as matrix material and oil phase, respectively.

In general, the correlation between interfacial and emulsion properties is well accepted (Murray & Dickinson, 1996). A viscoelastic protein film is known to preserve the stability of spray dried emulsions in oil droplet size and encapsulation efficiency during processing steps (Vega & Roos, 2006), and highly interfacial active low molecular weight emulsifiers are known to reduce the oil droplet size (Talón et al., 2019; Wilde et al., 2004). Such a highly viscoelastic film with strong intermolecular interactions (**Figure III-5**, page 33) with a low phase angle and thus high elastic interfacial reaction (**Table IV-3**, page 52) was formed by β-lactoglobulin as model protein for whey protein. The viscoelasticity of β-lactoglobulin was increased by protein enrichment at the interface caused by the excluded volume effect between β-lactoglobulin and starch conversion products in the water phase (Baeza et al., 2004; Rodríguez Patino & Pilosof, 2011), as discussed in **Chapter III.** During spray drying experiments, the oil droplet size of the whey protein stabilized feed emulsion is comparably high and is reduced during the atomization step (**Chapter III.4** from page 34). So, the interfacial film was partly disrupted during atomization, but preserved the oil droplet size and thus

encapsulation efficiency during atomization and subsequent drying step, resulting in comparably stable spray dried emulsions **Chapter III.3.1, III.4 and IV.3.3** from page 27, 34 and 51.

In comparison, the performance of combined emulsifying constituents during processing steps were affected by more complex interactions. The mixed interfacial films experienced common mechanisms like protein displacement (Bos & van Vliet, 2001) and non-attractive interactions (Lam & Nickerson, 2013), causing a reduction in viscoelasticity and intermolecular interactions for β-lactoglobulin and lecithin, mono-/diglyceride or citrem stabilized interfacial films (**Figure III-5**, page 33), as outlined in **Chapter III.4 and IV.3.3** from page 34 and 51. Since a viscoelastic interfacial film preserves the stability of spray dried emulsions (Vega & Roos, 2006), all mixed interfacial films were more easily disrupted during emulsification and atomization steps. Disrupted interfacial films were stabilized fast by the emulsifying constituent combination of lecithin and whey protein with the lowest interfacial tension and highest interfacial activity, avoiding oil droplet coalescence and growth of oil droplet size. The disrupted oil droplets are stabilized less fast via whey protein with addition of mono-/diglyceride or citrem, leading to an increase in oil droplet size by coalescence (**Chapter III.4** from page 34).

During the drying step, the oil droplets are getting in closer contact to each other due to water evaporation, volume reduction and viscosity increase. An interfacial film with a high elastic interfacial reaction preserved the oil droplet size and thus the encapsulation efficiency by preventing coalescence. This interfacial film was stabilized with β-lactoglobulin and lecithin (**Table IV-3**, page 52). Films with less elastic interfacial reaction like β-lactoglobulin with citrem or mono-/diglyceride led to an increase in oil droplet size via coalescence (**Chapter IV.3.3** from page 51). In general, it is notable that the lecithin with unsaturated fatty acids operates more effectively at the interface than the mono-/diglyceride and citrem with saturated fatty acids.

The described mechanisms illustrate the impact of the interactions of formulation components on the stability of spray dried emulsions during processing steps in liquid state. This gives guidance to sensibly tailor the emulsifying constituent combinations. Selection criteria might be: a protein with a capability to form a highly viscoelastic film at the pH of the system, and a LMWE with a high interfacial activity, namely interfacial tension, and with presumably unsaturated fatty acids. However, the presented data

gave indication that the interactions between protein and starch conversion products in the water phase modify the interfacial properties. Furthermore, since the molecular structure of the low molecular weight emulsifiers affected the interfacial properties, the oil phase might also be part of this mechanism. It is assumed that the interactions between protein and starch conversion products, and between oil and low molecular weight emulsifiers depend on the molecular structure and affect the performance of emulsifying constituents. Therefore, the following chapters will focus on the complex interplay of formulation components in the water and oil phase. The gathered knowledge might help to understand mechanisms in previous studies. For instance, a previous study showed that addition of lecithin to a sodium caseinate stabilized spray dried emulsion led to a lower oil droplet size and to a higher encapsulation efficiency of the anhydrous milk fat in comparison to the addition of monoglyceride (Danviriyakul et al., 2002). Yet another study showed that a spray dried emulsion with peony seed oil stabilized with whey protein increased the encapsulation efficiency with the addition of soy lecithin (Shujie Wang et al., 2017).

VII.2 Impact of interactions of formulation components in the water phase on the performance of emulsifying constituents

Building on the knowledge of the previous chapter, the interactions of formulation components in the water phase shall be systemized with interfacial modulation. The systemized knowledge might help to tailor the components of the water phase to improve the performance of the emulsifying constituents during processing steps in the liquid state.

In the water phase, usually proteins and starch conversion products are solubilized. In general, proteins act as emulsifying constituent by moving from a water phase to an interface, adsorbing at the interface and forming a viscoelastic layer with intermolecular interactions (Dickinson, 2011; Murray & Dickinson, 1996; Yampolskaya & Platikanov, 2006). The interfacial stabilization of proteins is affected by the viscosity increase and thermodynamic incompatibility effects of starch conversion products or any other neutral carbohydrate solubilized in the water phase.

The results presented in **chapter V** page 56ff. are focusing on β-lactoglobulin with the addition of application-oriented concentrations of maltodextrin and glucose syrup with an increasing dextrose equivalent from 9 to 14 and 37. In fact, the discussed correlations may easily be transferred to other emulsion systems with proteins and

neutral carbohydrates at high dry matter content solubilized in the water phase, as long as the prerequisite of the underlying physical mechanisms are met. The protein movement to the interface is decelerated by the viscosity increase of the water phase (**Figure V-3**, page 71), as described in the Stokes Einstein equation as one part of the Ward Tordai theory (Ward & Tordai, 1946). The viscosity increased with decreasing dextrose equivalent of starch conversion products, increasing molecular size and concentration (**Table V-1**, page 69), which belongs to the well accepted knowledge (Dokic et al., 1998). The adsorbed protein lowered the interfacial tension to a higher extent if starch conversion products with decreasing dextrose equivalent are solubilized in the water phase (**Figure V-4**, page 73). This effect is based on the thermodynamic incompatibility of protein and neutral carbohydrates (Antipova & Semenova, 1997; Baeza et al., 2004, 2005; Perez et al., 2010). The thermodynamic incompatibility of protein and neutral carbohydrates is increasing with increasing difference in molecular weight (Semenova & Dickinson, 2010) and is especially pronounced for globulins and neutral polysaccharide mixtures at high concentrations with a pH above the isoelectric point of the protein (Grinberg & Tolstoguzov, 1997). The thermodynamic incompatibility, or also called excluded volume effect, coincides with a protein enrichment close to the interface (Rodríguez Patino & Pilosof, 2011) and causes a change of the interfacial film viscoelasticity towards a more elastic behaviour (Baeza et al., 2004; Perez et al., 2010). In our case, the viscoelasticity of the β-lactoglobulin film shifted to a more elastic behaviour with addition of starch conversion products, independent of the molecular size and dextrose equivalent (**Figure V-5**, page 75). The elastic behaviour might be identified with linear Lissajous-plots and decreases in phase angle for dilatational rheological investigations (**Figure V-6**, page 76). The increase in elastic behaviour was based on the increase in intermolecular interactions in densely packed films (**Figure V-7**, page 77), which tend to be intramolecular β-sheets between the β-lactoglobulin molecules, as described earlier (Schestkowa et al., 2020). In our case, we assume a higher density in intramolecular β-sheets connecting the β-lactoglobulin molecules (**Chapter V.3.4**, page 72).

The interactions of proteins and starch conversion products in the water phase generally improve the performance of emulsifying constituents. For β-lactoglobulin, it was shown that, independent of molecular weight or dextrose equivalent of starch conversion products, the interfacial tension was reduced and the film viscoelasticity

was increased. Thus, it is assumed that the impact of the matrix material on the performance of milk proteins during processing steps is constant for all starch conversion products. This assumption is based on the prerequisites for thermodynamic incompatibility of proteins and neutral carbohydrates (Grinberg & Tolstoguzov, 1997). The prerequisites which always apply are: the starch conversion products are used at a high concentration (Danviriyakul et al., 2002; Drapala et al., 2017; Masum et al., 2019), and the pH of the water phase is above the isoelectric point of most milk proteins with 6.8 to 6.6 for infant formula (Drapala et al., 2017; Masum et al., 2019; McCarthy et al., 2012, 2015) or with 5.5 to 7 for coffee creamer (Kraft Foods Inc. & Zeller, 1997). The isoelectric point of milk proteins ranges between a pH of 4.3 to 5.6 up to 8.5 (Tavares et al., 2014). Since lactose is another common matrix material for spray dried emulsions (McCarthy et al., 2012, 2015), further studies should investigate the impact of lactose in application oriented concentrations on the performance of emulsifying constituents. It is assumed that the mechanism will be different, since mono- and disaccharides are known to act as conformational stabilizers of proteins (Shukla et al., 2011) and are not known to exhibit thermodynamic incompatibility with proteins.

VII.3 Impact of interactions of formulation components in the oil phase on the performance of emulsifying constituents and on physicochemical mechanisms during storage

Following the previous chapters, the interactions of formulation components in the oil phase shall be systemized with interfacial modulation. This knowledge represents the final step to add a puzzle piece to the big picture of the complex interplay of formulation components affecting the performance of emulsifying constituents like proteins and LMWE during processing steps in the liquid state. Further, the effect of the interactions between oil phase and LMWE on physicochemical mechanisms during storage shall be discussed.

Beside the earlier presented details, the interfacial stabilization of LMWEs is affected by interactions with the oil phase (Hildebrandt et al., 2016) and interactions with proteins situated at the interface (Murray & Dickinson, 1996). The results presented in the previous chapters are focusing on β-lactoglobulin with addition of commercial lecithin, mono-/diglyceride and citrem (**Chapter III and IV** from pages 18 and 39), or with addition of phosphatidylcholines with mainly un- or saturated fatty acids (**Chapter**

VI from pages 80). In most cases, the LMWEs are solubilized in oil phases with mainly saturated fatty acids (**Chapter III, IV and VI**), but also with mainly unsaturated fatty acids (**Chapter VI**). In all the described cases, we assumed coexistence of β-lactoglobulin and LMWE with two different experimental setups as outlined in **Chapter III.2.9, IV.2.4 and VI.3.2** at page 26, 46 and 92.

The movement of LMWEs to the interface is affected by interactions with the oil phase (Hildebrandt et al., 2016). These interactions are dispersion forces or π-interactions (Belitz et al., 2009; Walstra, 2003), which are increasing with increasing similarity in fatty acid composition of LMWEs and oil phase (Garti & Yano, 2001). For phospholipids, strong interactions with the oil phase are decelerating the adsorption to the interface and the reduction in interfacial tension (**Chapter VI.3.1** from page 90). This mechanism is also valid if proteins like β-lactoglobulin share the interface with LMWEs. The reduction in interfacial tension of a β-lactoglobulin and LMWE stabilized interface is decelerated by strong interactions of saturated fatty acids in oil phase, and mono-/diglyceride, citrem or phosphatidylcholine. This oil phase with its tightly packed, saturated fatty acids interacts less strongly with lecithin and phosphatidylcholine with unsaturated fatty acids, leading to a stronger reduction in interfacial tension with an LMWE enrichment at the interface (**Table IV-3** and **Figure VI-4** at page 52 and 93; **Chapter IV.3.3** from page 51 and **Chapter VI.3.2** from page 92). These effects are reversed in an oil phase with mainly unsaturated, less tightly packed and kinked fatty acids. A phosphatidylcholine with saturated fatty acids reduced the interfacial tension of a β-lactoglobulin and phosphatidylcholine stabilized interface stronger than a phosphatidylcholine with mainly unsaturated fatty acids (**Figure VI-5** at page 94 and **Chapter VI.3.3** from page 93).

The properties of the β-lactoglobulin and LMWE stabilized interfacial films are also affected by interactions of LMWEs with the oil phase. Since low interactions between LMWE and oil phase are causing a LMWE enrichment at the interface with a higher reduction in interfacial tension, the LMWE enrichment leads to a more responsive interfacial reaction with a low phase angle and a high elastic modulus of the β-lactoglobulin and LMWE stabilized interfacial films. This phenomenon is valid for lecithin or phosphatidylcholine with unsaturated fatty acid chains situated in an oil phase with saturated fatty acids (**Table IV-3**, **Figure VI-6** and **Figure VI-7** at page 52, 96 and 97 **Chapter IV.3.3** from page 51, **Chapter VI.3.4** from page 95). The reversed

effect was shown for strongly interacting saturated fatty acid chains of oil phase, phosphatidylcholine, mono-/diglyceride and citrem. The strong interactions between LMWE and oil phase are lowering the concentration of LMWEs close to the interface, which causes a high phase angle for these crystallized LMWEs, as well as a less elastic and rigid behaviour of the β-lactoglobulin and LMWE stabilized films. A rigid interfacial film loses elastic portions with increasing stress during expansion and compression of the interfacial area (Rodríguez Patino, Rodríguez Nino, et al., 2001). (**Table IV-3**, **Figure VI-6** and **Figure VI-7** at page 52, 96 and 97 **Chapter IV.3.3** from page 51, **Chapter VI.3.4** from page 95).

Similar effects were observed for an oil phase with mainly unsaturated fatty acids. Strong interactions between phosphatidylcholine and an oil phase with mainly unsaturated fatty acids decreased the LMWE concentration at the interface, reducing the interfacial reactivity and leading to a higher phase angle and lower film elasticity. Less interactions between oil phase with mainly unsaturated fatty acids and crystallized phosphatidylcholine with saturated fatty acids caused a high phase angle, but a high and rigid elastic behaviour of the β-lactoglobulin and LMWE stabilized interfacial film (**Figure VI-8** and **Figure VI-9** at page 98 and 99; **Chapter VI.3.5** from page 97).

In addition, the interactions and the crystallization behaviour of LMWE and oil phase affect the physicochemical mechanisms during storage time, as well. Beside the aforementioned characteristic of crystallized LMWE to induce a rigid film behaviour, the crystallization of LMWE at the interface may be also linked to surface heterogenous nucleation (McClements, 2012; Ribeiro et al., 2015). The crystals of the surface heterogenous nucleation may undergo a polymorphic transition from the spherical α crystal with the lowest activation energy to the needle-like β crystal with a lower free energy (Awad et al., 2008; McClements, 2012). These crystals may pierce through the interface and damage the interfacial barrier as described for liquid emulsions (Fredrick et al., 2013; Goibier et al., 2017). This interfacial damage via fat crystals may lead to a release of encapsulated oil in spray dried emulsions (Fäldt & Bergenståhl, 1995; E. H. J. Kim et al., 2005; Millqvist-Fureby, 2003). This mechanism has been described in **Chapter III** from page 34 for emulsion systems with mono-/diglyceride and citrem with mainly saturated fatty acids. The interfacial films composed of whey protein, and whey protein and lecithin with unsaturated fatty acids gave no evidence for such phenomena.

These results present a high potential to enhance the performance of emulsifying constituents during processing steps in the liquid state and to guide the physicochemical mechanisms during storage, leading to an improved stability of spray dried emulsions. A LMWE which is interacting less with the oil phase reduced the interfacial tension and increased the viscoelasticity of the protein and LMWE stabilized interfacial films. Such a combination of formulation components may strongly improve the performance of emulsifying constituents during processing steps. In contrast, crystallization of LMWE may change the interfacial properties to a more rigid interfacial behaviour. Since such a crystallized LMWE may cause physicochemical mechanisms during storage time of spray dried emulsions, such an interfacial film destabilized spray dried emulsions with release of encapsulated oil. Further interfacial and spray dried emulsion studies might help to combine the facts and to improve the comprehension.

VIII. Conclusion

Spray drying of emulsions is a well-established method which has been investigated in uncountable publications. Nevertheless, some research questions have not been addressed in the past. Beside the impact of processing, the earlier presented research **(Chapter III, IV, V and VI)** focused mainly on the impact of formulation on the characteristics and stability of spray dried emulsions. The complex interplay of formulation components altered the performance of emulsifying constituents, affecting the oil droplet size and encapsulation efficiency of spray dried emulsions during processing steps before powder formation and the physicochemical mechanisms during storage time.

The performance of emulsifying constituents is high if the interfacial film has a high viscoelasticity and a low interfacial tension. A high viscoelasticity and a low interfacial tension led to a reduced oil droplet size in emulsions and spray dried emulsions during emulsification, atomization and drying step. Such an interfacial film may be composed of a protein surrounded by starch conversion products in the water phase, and a LMWE with a high interfacial activity and unsaturated fatty acids which are insoluble in the oil phase. Thus, the performance of emulsifying constituents is increased via the excluded volume effect between proteins and neutral carbohydrates in the water phase and via low interactions between LMWE and oil phase. In contrast, a protein and a LMWE stabilized interfacial film with a rigid behaviour and a higher interfacial tension may cause oil droplet coalescence and may decrease encapsulation efficiency during processing steps. Thus, the performance of emulsifying constituents is decreased via high interactions between LMWE and oil phase and crystallization behaviour of LMWE.

During storage time, the formulation components undergo physicochemical mechanisms, affecting the stability of spray dried emulsions. It was shown that the surface heterogenous nucleation of LMWE may cause a release of encapsulated oil via polymorphic phase transition, leading to needle like emulsifier crystals damaging the interfacial film.

The connections between properties of spray dried emulsion and interfaces illustrates and clarifies the importance and necessity of their simultaneous consideration. The use of new and emerging techniques will increase the comprehension of the impact of the complex interplay of formulation components on interfacial performance during

processing steps and will potentially explain the physicochemical mechanisms of LMWE and oil phase during storage. These techniques are SANS, SAXS, NMR, grazing incidence X-ray diffraction, interfacial microrheology, single particle Brownian dynamics and thermo optical microscopy (Abramov et al., 2016; Bernewitz et al., 2011; Ghazvini et al., 2015; Gilbert, 2019; Jaksch et al., 2019; Oberdisse & Hellweg, 2017; Park et al., 2016; Yesiltas et al., 2019). These emerging techniques will help to investigate the arrangement of emulsifying constituents in interfacial films and will help to identify crystallization events of LMWE at interfaces and in emulsions. They will also provide details about the impact of crystal size, form and polymorphism on interfacial and emulsion characteristics, depending on the applied temperature. The upcoming mechanistic knowledge will assist with tailoring the formulation of emulsions and spray dried emulsions, leading to extended and improved stability for food application.

IX. References

Aalaei, K., Rayner, M., & Sjöholm, I. (2019). Chemical methods and techniques to monitor early Maillard reaction in milk products; A review. *Critical Reviews in Food Science and Nutrition*, *59*, 1829–1839. https://doi.org/10.1080/10408398.2018.1431202

Abramov, S., Ruppik, P., & Schuchmann, H. P. (2016). Crystallization in emulsions: A thermo-optical method to determine single crystallization events in droplet clusters. *Processes*, *4*(3), 1–13. https://doi.org/10.3390/pr4030025

Antipova, A. S., & Semenova, M. G. (1995). Effect of sucrose on the thermodynamic incompatibility of different biopolymers. *Carbohydrate Polymers*, *28*(4), 359–365. https://doi.org/10.1016/0144-8617(96)00002-1

Antipova, A. S., & Semenova, M. G. (1997). Effect of neutral carbohydrate structure in the set glucose/sucrose/maltodextrin/dextran on protein surface activity at the air/water interface. *Food Hydrocolloids*, *11*(1), 71–77. https://doi.org/10.1016/S0268-005X(97)80013-6

Arranz, E., & Corredig, M. (2017). Invited review: Milk phospholipid vesicles, their colloidal properties, and potential as delivery vehicles for bioactive molecules. *Journal of Dairy Science*, *100*(6), 4213–4222. https://doi.org/10.3168/jds.2016-12236

Awad, T. S., Helgason, T., Kristbergsson, K., Decker, E. A., Weiss, J., & McClements, D. J. (2008). Effect of cooling and heating rates on polymorphic transformations and gelation of tripalmitin Solid Lipid Nanoparticle (SLN) suspensions. *Food Biophysics*, *3*, 155–162. https://doi.org/10.1007/s11483-008-9057-8

Baeza, R., Carrera Sanchez, C., Pilosof, A. M. R., & Rodríguez Patino, J. M. (2004). Interactions of polysaccharides with β-lactoglobulin spread monolayers at the air-water interface. *Food Hydrocolloids*, *18*(6), 959–966. https://doi.org/10.1016/j.foodhyd.2004.03.006

Baeza, R., Carrera Sanchez, C., Pilosof, A. M. R., & Rodríguez Patino, J. M. (2005). Interactions of polysaccharides with β-lactoglobulin adsorbed films at the air-water interface. *Food Hydrocolloids*, *19*(2), 239–248. https://doi.org/10.1016/j.foodhyd.2004.06.002

Bakry, A. M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M. Y., Mousa, A., & Liang, L. (2016). Microencapsulation of oils: A comprehensive review of benefits, techniques, and applications. *Comprehensive Reviews in Food Science and Food Safety*, *15*(1), 143–182. https://doi.org/10.1111/1541-4337.12179

Barbosa-Cánovas, G. V, Ortega-Rivas, E., Juliano, P., & Yan, H. (2005). *Food powders - physical properties, processing, and functionality*. Kluwer Academic Plenum Publishers New York.

Belitz, H. D., Grosch, W., & Schieberle, P. (2009). *Food chemistry*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-69934-7

Bergfreund, J., Bertsch, P., & Fischer, P. (2021). Adsorption of proteins to fluid interfaces: Role of the hydrophobic subphase. *Journal of Colloid and Interface Science*, *584*(October), 411–417. https://doi.org/10.1016/j.jcis.2020.09.118

Bergfreund, J., Bertsch, P., Kuster, S., & Fischer, P. (2018). Effect of oil hydrophobicity on the adsorption and rheology of β-Lactoglobulin at oil-water interfaces. *Langmuir*, *34*(16), 4929–4936. https://doi.org/10.1021/acs.langmuir.8b00458

Bergfreund, J., Siegenthaler, S., Lutz-Bueno, V., Bertsch, P., & Fischer, P. (2021). Surfactant adsorption to different fluid interfaces. *Langmuir*, *37*(22), 6722–6727. https://doi.org/10.1021/acs.langmuir.1c00668

Bernewitz, R., Guthausen, G., & Schuchmann, H. P. (2011). NMR on emulsions: characterisation of liquid dispersed systems. *Magnetic Resonance in Chemistry*, *49*, S93–S104. https://doi.org/10.1002/mrc.2825

Berry, J. D., Neeson, M. J., Dagastine, R. R., Chan, D. Y. C., & Tabor, R. F. (2015). Measurement of surface and interfacial tension using pendant drop tensiometry. *Journal of Colloid and Interface Science*, *454*, 226–237. https://doi.org/10.1016/j.jcis.2015.05.012

Bertsch, P., Diener, M., Adamcik, J., Scheuble, N., Geue, T., Mezzenga, R., & Fischer, P. (2018). Adsorption and interfacial layer structure of unmodified nanocrystalline cellulose at air/water interfaces. *Langmuir*, *34*(50), 15195–15202. https://doi.org/10.1021/acs.langmuir.8b03056

Bertsch, P., Diener, M., Adamcik, J., Scheuble, N., Geue, T., Mezzenga, R., & Fischer, P. (2020). Correction to adsorption and interfacial layer structure of unmodified nanocrystalline cellulose at air/water interfaces. *Langmuir*, *36*(7), 1848–1849. https://doi.org/10.1021/acs.langmuir.0c00263

Bertsch, P., & Fischer, P. (2019). Interfacial rheology of charged anisotropic cellulose nanocrystals at the air–water interface. *Langmuir*, *35*, 7937–7943. https://doi.org/10.1021/acs.langmuir.9b00699

Bertsch, P., & Fischer, P. (2020). Correction to “ interfacial rheology of charged anisotropic cellulose nanocrystals at the air − water interface .” *Langmuir*, *36*, 3676. https://doi.org/10.1021/acs.langmuir.0c00715

Blanchard, P. H., & Katz, F. R. (2006). *Starch hydrolysates in Food polysaccharides and their applications, second edition* (A. M. Stephen, G. O. Phillips, & P. A. Williams (eds.)). CRC Press.

Boode, K., Bisperink, C., & Walstra, P. (1991). Destabilization of O/W emulsions containing fat crystals by temperature cycling. *Colloids and Surfaces*, *61*, 55–74. https://doi.org/10.1016/0166-6622(91)80299-4

Bos, M. A., & van Vliet, T. (2001). Interfacial rheological properties of adsorbed protein layers and surfactants: a review. *Advances in Colloid and Interface Science*, *91*, 437–471. https://doi.org/10.1016/S0001-8686(00)00077-4

Böttcher, S., Keppler, J. K., & Drusch, S. (2017). Mixtures of Quillaja saponin and beta-lactoglobulin at the oil/water-interface: Adsorption, interfacial rheology and emulsion properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *518*, 46–56. https://doi.org/10.1016/j.colsurfa.2016.12.041

Brinkmann, C. R., Thiel, S., & Otzen, D. E. (2013). Protein-fatty acid complexes: biochemistry, biophysics and function. *FEBS Journal*, *280*, 1733–1749. https://doi.org/10.1111/febs.12204

Burgos-Diaz, C., Opazo-Navarrete, M., Soto-Anual, M., Leal-Calderon, F., & Bustamante, M. (2020). Food-grade Pickering emulsion as novel astaxanthin encapsulation system for making powder-based products: Evaluation of asaxanthin stabilty during processing storage, and its bioaccessibility. *Food Research International*, *134*, 109244. https://doi.org/10.1016/j.foodres.2020.109244

Bylaite, E., Nylander, T., Venskutonis, R., & Jönsson, B. (2001). Emulsification of caraway essential oil in water by lecithin and β-lactoglobulin: Emulsion stability and properties of the formed oil-aqueous interface. *Colloids and Surfaces B: Biointerfaces*, *20*(4), 327–340. https://doi.org/10.1016/S0927-7765(00)00212-5

Cal, K., & Solohub, K. (2010). Spray drying technique. I: Hardware and process parameters. *Journal of Pharmaceutical Science*, *99*, 575–586. https://doi.org/10.1002/jps.21886

Campbell, R. A., Saaka, Y., Shao, Y., Gerelli, Y., Cubitt, R., Nazaruk, E., Matyszewska, D., & Lawrence, M. J. (2018). Structure of surfactant and phospholipid monolayers at the air/water interface modeled from neutron reflectivity data. *Journal of Colloid and Interface Science*, *531*, 98–108. https://doi.org/10.1016/j.cis.2018.07.022

Chen, J., & Dickinson, E. (1995). Surface shear viscosity and protein-surfactant interactions in mixed protein films adsorbed at the oil-water interface. *Food Hydrocolloids*, *9*(1), 35–42. https://doi.org/10.1016/S0268-005X(09)80191-4

Cholakova, D., Denkov, N., Tcholakova, S., Lesov, I., & Smoukov, S. K. (2016). Control of drop shape transformations in colled emulsions. *Advances in Colloid and Interface Science*, *235*, 90–107. https://doi.org/10.1016/j.cis2016.06.002

Cholakova, D., Denkov, N., Tcholakova, S., Valkova, Z., & Smoukov, S. K. (2019). Multilayer formation in self-shaping emulsion droplets. *Langmuir*, *35*, 5484–5495. https://doi.org/10.1021/acs.langmuir.8b02771

Crespo-Villanueva, A., Gumí-Audenis, B., Sanz, F., Artzner, F., Mériadec, C., Rousseau, F., Lopez, C., Giannotti, M. I., & Guyomarc'h, F. (2018). Casein interaction with lipid membranes: are the phase state or charge density of the phospholipids affecting protein adsorption? *Biochimica et Biophysica Acta - Biomembranes*, *1860*, 2588–2598. https://doi.org/10.1016/j.bbamem.2018.09.016

Cui, L., & Decker, E. A. (2016). Phospholipids in foods: prooxidants or antioxidants? *Journal of the Science of Food and Agriculture*, *96*, 18–31. https://doi.org/10.1002/jsfa.7320

Cuq, B., Rondet, E., & Abecassis, J. (2011). Food powders engineering, between knowhow and science: constraints, stakes and opportunities. *Powder Technology*, *208*, 244–251. https://doi.org/10.1016/j.powtec.2010.08.012

Dan, A., Gochev, G., Krägel, J., Aksenenko, E. V., Fainerman, V. B., & Miller, R. (2013). Interfacial rheology of mixed layers of food proteins and surfactants. *Current Opinion in Colloid and Interface Science*, *18*, 302–310. https://doi.org/10.1016/j.cocis.2013.04.002

Danviriyakul, S., McClements, D. J., Decker, E., Nawar, W. W., & Chinachoti, P. (2002). Physical stability of spray-dried milk fat emulsion as affected by emulsifiers and processing conditions. *Journal of Food Science*, *67*(6), 2183–2189. https://doi.org/10.1111/j.1365-2621.2002.tb09524.x

de Kruif, C. G., & Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids*, *15*, 555–563. https://doi.org/10.1016/S0268-005X(01)00076-5

de Souza Lima, R., Ré, M. I., & Arlabosse, P. (2020). Drying droplet as a template for solid formation: A review. *Powder Technology*, *359*, 161–171. https://doi.org/10.1016/j.powtec.2019.09.052

Delshadi, R., Bahrami, A., Tafti, A. G., Barba, F. J., & Williams, L. L. (2020). Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. *Trends in Food Science & Technology*, *104*, 72–83. https://doi.org/10.1016/j.tifs.2020.07.004

Denkov, N., Tcholakova, S., & Cholakova, D. (2019). Surface phase transitions in foams and emulsions. *Current Opinon in Colloid and Interface Science*, *44*, 32–47. https://doi.org/10.1016/j.cocis.2019.09.005

Denkov, N., Tcholakova, S., Lesov, I., Cholakova, D., & Smoukov, S. K. (2015). Self-shaping of oil droplets via the formation of intermediate rotator phases upon cooling. *Nature*, *528*(7582), 392–395. https://doi.org/10.1038/nature16189

Di Cola, E., Torbensen, K., Clemente, I., Rossi, F., Ristori, S., & Abou-Hassan, A. (2017). Lipid-stabilized water-oil interfaces studied by microfocusing small-angle X-ray scattering. *Langmuir*, *33*(36), 9100–9105. https://doi.org/10.1021/acs.langmuir.7b02076

Dickinson, E. (2011). Mixed biopolymers at interfaces: Competitive adsorption and multilayer structures. *Food Hydrocolloids*, *25*(8), 1966–1983. https://doi.org/10.1016/j.foodhyd.2010.12.001

Dokic, P., Jakovljevic, J., & Dokic-Baucal, L. (1998). Molecular characteristics of maltodextrins and rheological behaviour of diluted and concentrated solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *141*(3), 435–440. https://doi.org/10.1016/S0927-7757(97)00118-0

Douaire, M., Di Bari, V., Norton, J. E., Sullo, A., Lillford, P., & Norton, I. T. (2014). Fat crystallisation at oil-water interfaces. *Advances in Colloid and Interface Science*, *203*, 1–10. https://doi.org/10.1016/j.cis.2013.10.022

Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2015). Influence of lecithin on the processing stability of model whey protein hydrolysate-based infant formula emulsions. *International Journal of Dairy Technology*, *68*(3), 322–333. https://doi.org/10.1111/1471-0307.12256

Drapala, K. P., Auty, M. A. E., Mulvihill, D. M., & O'Mahony, J. A. (2017). Influence of emulsifier type on the spray-drying properties of model infant formula emulsions. *Food Hydrocolloids*, *69*, 56–66. https://doi.org/10.1016/j.foodhyd.2016.12.024

Drusch, S., & Berg, S. (2008). Extractable oil in microcapsules prepared by spray-drying: Localisation, determination and impact on oxidative stability. *Food Chemistry*, *109*(1), 17–24. https://doi.org/10.1016/j.foodchem.2007.12.016

Duckworth, R. B. (1975). Water relations of foods. *Food Science and Technology. Academic Press, London*.

Engelhardt, K., Lexis, M., Gochev, G., Konnerth, C., Miller, R., Willenbacher, N., Peukert, W., & Braunschweig, B. (2013). PH effects on the molecular structure of β-lactoglobulin modified air-water interfaces and its impact on foam rheology. *Langmuir*, *29*(37), 11646–11655. https://doi.org/10.1021/la402729g

Erni, P., Fischer, P., Windhab, E. J., Kusnezov, V., Stettin, H., & Läuger, J. (2003). Stress- and strain-controlled measurements of interfacial shear viscosity and viscoelasticity at liquid/liquid and gas/liquid interfaces. *Review of Scientific Instruments*, *74*(11), 4916–4924. https://doi.org/10.1063/1.1614433

European Communities. (2016). *Infant formulae and follow-on formulae, Amendment Regulation 2016/127/EG and 2006/141/EG* (Issue 4, pp. 1–31). .

Fäldt, P., & Bergenståhl, B. (1995). Fat encapsulation in spray-dried food powders. *JAOCS, Journal of the American Oil Chemists' Society*, *72*(2), 171–176. https://doi.org/10.1007/BF02638895

Fang, S., Zhao, X., Liu, Y., Liang, X., & Yang, Y. (2019). Fabricating multilayer emulsions by using OSA starch and chitosan suitable for spray drying: Application in the encapsulation of β-carotene. *Food Hydrocolloids*, *93*, 102–110. https://doi.org/10.1016/j.foodhyd.2019.02.024

Foegeding, E. A., Luck, P., & Vardhanabhuti, B. (2011). *Milk protein products - Whey protein products. In J.W. Fuquay (2nd Ed.), Encyclopedia of dairy sciences*. Academic Press.

Fredrick, E., Heyman, B., Moens, K., Fischer, S., Verwijlen, T., Moldenaers, P., Van der Meeren, P., & Dewettinck, K. (2013). Monoacylglycerols in dairy recombined cream: II. the effect on partial coalescence and whipping properties. *Food Research International*, *51*, 936–945. https://doi.org/10.1016/j.foodres.2013.02.006

Freer, E. M., Wong, H., & Radke, C. J. (2005). Oscillating drop/bubble tensiometry: Effect of viscous forces on the measurement of interfacial tension. *Journal of Colloid and Interface Science*, *282*(1), 128–132. https://doi.org/10.1016/j.jcis.2004.08.058

Garti, N., & Sato, K. (Eds). (2001). *Crystallization processes in fats and lipid systems*. Marcel Dekker, Inc; New York, USA; Basel, Switzerland.

Garti, N., & Yano, J. (2001). The roles of emulsifiers in fat crystallization. In N. Garti & K. Sato (Eds.), *Crystallization processes in fats and lipid systems* (p. 211 ff.). Marcel Dekker, Inc; New York, USA; Basel, Switzerland.

Geranpour, M., Assadpour, E., & Jafari, S. M. (2020). Recent advances in the spray drying encapsulation of essential fatty acids and functional oils. *Trends in Food Science & Technology*, *102*, 71–90. https://doi.org/10.1016/j.tifs.2020.05.028

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, *40*(9), 1107–1121. https://doi.org/10.1016/j.foodres.2007.07.004

Gharsallaoui, A., Saurel, R., Chambin, O., Cases, E., Voilley, A., & Cayot, P. (2010). Utilisation of pectin coating to enhance spray-dry stability of pea protein-stabilised oil-in-water emulsions. *Food Chemistry*, *122*, 447–454. https://doi.org/10.1016/j.foodchem.2009.04.017

Ghazvini, S., Alonso, R., Alhakamy, N., & Dhar, P. (2018). PH-induced changes in the surface viscosity of unsaturated phospholipids monitored using active interfacial microrheology. *Langmuir*, *34*(3), 1159–1170. https://doi.org/10.1021/acs.langmuir.7b02803

Ghazvini, S., Ricke, B., Zasadzinski, J. A., & Dhar, P. (2015). Monitoring phases and phase transitions in phosphatidylethanolamine monolayers using active interfacial microrheology. *Soft Matter*, *11*(17), 3313–3321. https://doi.org/10.1039/c4sm02900c

Gilbert, E. P. (2019). Small-angle X-Ray and neutron scattering in food colloids. *Current Opinion in Colloid and Interface Science*, *42*, 55–72. https://doi.org/10.1016/j.cocis.2019.03.005

Goibier, L., Lecomte, S., Leal-Calderon, F., & Faure, C. (2017). The effect of surfactant crystallization on partial coalescence in O/W emulsions. *Journal of Colloid and Interface Science*, *500*, 304–314. https://doi.org/10.1016/j.jcis.2017.04.021

Golding, M., & Sein, A. (2004). Surface rheology of aqueous casein-monoglyceride dispersions. *Food Hydrocolloids*, *18*, 451–461. https://doi.org/10.1016/j.foodhyd.2003.08.003

Grinberg, V. Y., & Tolstoguzov, V. B. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*(2), 145–158. https://doi.org/10.1016/S0268-005X(97)80022-7

Guttman, S., Sapir, Z., Ocko, B. M., Deutsch, M., & Sloutskin, E. (2017). Temperature-tuned faceting and shape changes in liquid alkane droplets. *Langmuir*, *33*(5), 1305–1314. https://doi.org/10.1021/acs.langmuir.6b02926

Haas, K., Obernberger, J., Zehetner, E., Kiesslich, A., Volkert, M., & Jaeger, H. (2019). Impact of powder particle structure on the oxidation stability and color of encapsulated crystalline and emulsified carotenoids in carrot concentrate powders. *Journal of Food Engineering*, *263*(May), 398–408. https://doi.org/10.1016/j.jfoodeng.2019.07.025

Håkansson, A. (2016). Experimental methods for measuring coalescence during emulsification - A critical review. *Journal of Food Engineering*, *178*, 47–59. https://doi.org/10.1016/j.jfoodeng.2016.01.006

Håkansson, A., Fuchs, L., Innings, F., Revstedt, J., Bergenståhl, B., & Trägårdh, C. (2010). Visual observations and acoustic measurements of cavitation in an experimental model of a high-pressure homogenizer. *Journal of Food Engineering*, *100*(3), 504–513. https://doi.org/10.1016/j.jfoodeng.2010.04.038

Håkansson, A., Fuchs, L., Innings, F., Revstedt, J., Trägårdh, C., & Bergenståhl, B. (2011). On flow-fields in a high pressure homogenizer and its implication on drop fragmentation. *Procedia Food Science*, *1*, 1353–1358. https://doi.org/10.1016/j.profoo.2011.09.200

Håkansson, A., & Hounslow, M. J. (2013). Simultaneous determination of fragmentation and coalescence rates during pilot-scale high-pressure homogenization. *Journal of Food Engineering*, *116*(1), 7–13. https://doi.org/10.1016/j.jfoodeng.2012.11.002

Håkansson, A., Innings, F., Trägårdh, C., & Bergenståhl, B. (2013). A high-pressure homogenization emulsification model-Improved emulsifier transport and hydrodynamic coupling. *Chemical Engineering Science*, *91*, 44–53. https://doi.org/10.1016/j.ces.2013.01.011

Håkansson, A., Trägårdh, C., & Bergenståhl, B. (2009). Dynamic simulation of emulsion formation in a high pressure homogenizer. *Chemical Engineering Science*, *64*(12), 2915–2925. https://doi.org/10.1016/j.ces.2009.03.034

Harkins, W. D., & Brown, F. E. (1919). The determination of surface tension (free surface energy), and the weight of falling drops: The surface tension of water and benzene by the capillary height method. *Journal of the American Chemical Society*, *41*(4), 499–524. https://doi.org/10.1021/ja01461a003

Heiden-Hecht, T., & Drusch, S. (2021). Impact of saturation of fatty acids of phospatidylcholine and oil phase on properties of β-lactoglobulin at the oil/water interface. *Food Biophysics*.

Heiden-Hecht, T., Taboada, M. L., Brückner-Gühmann, M., Karbstein, H. P., Gaukel, V., & Drusch, S. (2021). Towards an improved understanding of spray-dried emulsions: impact of the emulsifying constituent combination on characteristics and storage stability. *International Dairy Journal*, *121*, 105134. https://doi.org/10.1016/j.idairyj.2021.105134

Heiden-Hecht, T., Ulbrich, M., Drusch, S., & Brückner-Gühmann, M. (2021). Interfacial properties of β-lactoglobulin at the oil/water interface: influence of starch conversion products with varying dextrose equivalents. *Food Biophysics*, *16*, 169–180. https://doi.org/10.1007/s11483-020-09658-4

Hernandez Sanchez, M. D. R., Cuvelier, M. E., & Turchiuli, C. (2015). Design of liquid emulsions to structure spray dried particles. *Journal of Food Engineering*, *167*, 99–105. https://doi.org/10.1016/j.jfoodeng.2015.07.036

Hildebrandt, E., Dessy, A., Sommerling, J. H., Guthausen, G., Nirschl, H., & Leneweit, G. (2016). Interactions between phospholipids and organic phases: Insights into lipoproteins and nanoemulsions. *Langmuir*, *32*(23), 5821–5829. https://doi.org/10.1021/acs.langmuir.6b00978

Holder, C. F., & Schaak, R. E. (2019). Tutorial on powder X-ray diffraction for characterizing nanoscale materials. *ACS Nano*, *13*(7), 7359–7365. https://doi.org/10.1021/acsnano.9b05157

Hu, Y. T., Ting, Y., Hu, J. Y., & Hsieh, S. C. (2017). Techniques and methods to study functional characteristics of emulsion systems. *Journal of Food and Drug Analysis*, *25*(1), 16–26. https://doi.org/10.1016/j.jfda.2016.10.021

Hughes, D. J., Bönisch, G. B., Zwick, T., Schäfer, C., Tedeschi, C., Leuenberger, B., Martini, F., Mencarini, G., Geppi, M., Alam, M. A., & Ubbink, J. (2018). Phase separation in amorphous hydrophobically modified starch–sucrose blends: glass transition, matrix dynamics and phase behavior. *Carbohydrate Polymers*, *199*, 1–10. https://doi.org/10.1016/j.carbpol.2018.06.056

Jahan, R., Bodratti, A. M., Tsianou, M., & Alexandridis, P. (2020). Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Advances in Colloid and Interface Science*, *275*, 102061. https://doi.org/10.1016/j.cis.2019.102061

Jaksch, S., Gutberlet, T., & Müller-Buschbaum, P. (2019). Grazing-incidence scattering—status and perspectives in soft matter and biophysics. *Current Opinion in Colloid and Interface Science*, *42*, 73–86. https://doi.org/10.1016/j.cocis.2019.04.001

Joshi, A., Paratkar, S. G., & Thorat, B. N. (2006). Modification of lecithin by physical, chemical and enzymatic methods. *European Journal of Lipid Science and Technology*, *108*(4), 363–373. https://doi.org/10.1002/ejlt.200600016

Juárez, A. V., Juárez, A. V., Wilke, N., & Yudi, L. M. (2018). Combination of cyclic voltammetry and single-particle Brownian dynamics methodology to evaluate the fluidity of phospholipid monolayers at polarized liquid/liquid interfaces. *Electrochimica Acta*, *281*, 611–618. https://doi.org/10.1016/j.electacta.2018.05.118

Jung, J.-M., Gunes, D. Z., & Mezzenga, R. (2010). Interfacial activity and interfacial shear rheology of native β-lactoglobulin monomers and their heat-induced fibers. *Langmuir*, *26*(19), 15366–15375. https://doi.org/10.1021/la102721m

Keppler, J. K., Heyse, A., Scheidler, E., Uttinger, M. J., Fitzner, L., Jandt, U., Heyn, T. R., Lautenbach, V., Loch, J. I., Lohr, J., Kieserling, H., Günther, G., Kempf, E., Grosch, J. H., Lewiński, K., Jahn, D., Lübbert, C., Peukert, W., Kulozik, U., … Biedendieck, R. (2021). Towards recombinantly produced milk proteins: Physicochemical and emulsifying properties of engineered whey protein beta-lactoglobulin variants. *Food Hydrocolloids*, *110*(July 2020), 106132. https://doi.org/10.1016/j.foodhyd.2020.106132

Keppler, J. K., Sönnichsen, F. D., Lorenzen, P.-C., & Schwarz, K. (2014). Differences in heat stability and ligand binding among β-lactoglobulin genetic variants A , B and C using 1 H NMR and fluorescence quenching. *Biochimica et Biophysica Acta*, *1844*, 1083–1093. https://doi.org/10.1016/j.bbapap.2014.02.007

Kim, D. A., Cornec, M., & Narsimhan, G. (2005). Effect of thermal treatment on interfacial properties of β-lactoglobulin. *Journal of Colloid and Interface Science*, *285*(1), 100–109. https://doi.org/10.1016/j.jcis.2004.10.044

Kim, E. H. J., Chen, X. D., & Pearce, D. (2005). Melting characteristics of fat present on the surface of industrial spray-dried dairy powders. *Colloids and Surfaces B: Biointerfaces*, *42*(1), 1–8. https://doi.org/10.1016/j.colsurfb.2005.01.004

Kotsmar, C., Pradines, V., Alahverdjieva, V. S., Aksenenko, E. V, Fainerman, V. B., Kovalchuk, V. I., Krägel, J., Leser, M. E., Noskov, B. A., & Miller, R. (2009). Thermodynamics , adsorption kinetics and rheology of mixed protein – surfactant interfacial layers. *Advances in Colloid and Interface Science*, *150*, 41–54. https://doi.org/10.1016/j.cis.2009.05.002

Kraft Foods Inc., & Zeller, B. L. (1997). Foaming coffee creamer and instant cappuccino, EP 0 796 562 A2. In *European Patent Application*.

Krägel, J., Derkatch, S. R., & Miller, R. (2008). Interfacial shear rheology of protein-surfactant layers. *Advances in Colloid and Interface Science*, *144*, 38–53. https://doi.org/10.1016/j.cis.2008.08.010

Krog, N., & Larsson, K. (1992). Crystallization at interfaces in food emulsions - a general phenomenon. *Fat Science Technology*, *94*(2), 55–57. https://doi.org/10.1002/lipi.19920940205

Lam, R. S. H., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chemistry*, *141*(2), 975–984. https://doi.org/10.1016/j.foodchem.2013.04.038

Lam, R. S. H., & Nickerson, M. T. (2015). The effect of pH and temperature pre-treatments on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin. *Food Chemistry*, *173*, 163–170. https://doi.org/10.1016/j.foodchem.2014.09.078

Lee, H. O., Jiang, T. S., & Avramidis, K. S. (1991). Measurements of interfacial shear viscoelasticity with an oscillatory torsional viscometer. *Journal of Colloid And Interface Science*, *146*(1), 90–122. https://doi.org/10.1016/0021-9797(91)90009-W

Lefebvre, A. H., & Mcdonell, V. G. (2017). Atomization and sprays, second edition. In *CRC Press, Taylor and Francis*. https://doi.org/10.1201/9781315120911

Lerbret, A., Bordat, P., Affouard, F., Hédoux, A., Guinet, Y., & Descamps, M. (2007). How do trehalose, maltose, and sucrose influence some structural and dynamical properties of lysozyme? Insight from molecular dynamics simulations. *Journal of Physical Chemistry B*, *111*(31), 9410–9420. https://doi.org/10.1021/jp071946z

Lišková, K., Auty, M. A. E., Chaurin, V., Min, S., Mok, K. H., O'Brien, N., Kelly, A. L., & Brodkorb, A. (2011). Cytotoxic complexes of sodium oleate with β-lactoglobulin. *European Journal of Lipid Science and Technology*, *113*, 1207–1218. https://doi.org/10.1002/ejlt.201100109

Liu, Y., Wei, Z.-C., Deng, Y.-Y., Dong, H., Zhang, Y., Tang, X.-J., Li, P., Liu, G., & Zhang, M.-W. (2020). Comparison of the effects of different food-grade emulsifiers on the properties and stability of a casein-maltodextrin-soybean oil compound emulsion. *Molecules*, *25*(458), 1–17. https://doi.org/10.3390/molecules25030458

Loglio, G., Pandolfini, P., Miller, R., Makievski, A., Krägel, J., & Ravera, F. (2004). Oscillation of interfacial properties in liquid systems: Assessment of harmonic distortion. *Physical Chemistry Chemical Physics*, *6*(7), 1375–1379. https://doi.org/10.1039/b314592c

Lucassen-Reynders, E. H. (1993). Interfacial viscoelasticity in emulsions and foams. *Food Structure*, *12*(1), 1–12.

Lucassen-Reynders, E. H., Benjamins, J., & Fainerman, V. B. (2010). Dilational rheology of protein films adsorbed at fluid interfaces. *Current Opinion in Colloid and Interface Science*, *15*(4), 264–270. https://doi.org/10.1016/j.cocis.2010.05.002

Masum, A. K. M., Chandrapala, J., Adhikari, B., Huppertz, T., & Zisu, B. (2019). Effect of lactose-to-maltodextrin ratio on emulsion stability and physicochemical properties of spray-dried infant milk formula powders. *Journal of Food Engineering*, *254*, 34–41. https://doi.org/10.1016/j.jfoodeng.2019.02.023

Matsumiya, K., Takahashi, Y., Nakanishi, K., Dotsu, N., & Matsumura, Y. (2014). Diglycerol esters of fatty acids promote severe coalescence between protein-stabilized oil droplets by emulsifier-protein competitive interactions. *Food Hydrocolloids*, *42*, 397–402. https://doi.org/10.1016/j.foodhyd.2014.03.002

McCarthy, N. A., Gee, V. L., O'Mahony, J. A., Kelly, A. L., & Fenelon, M. A. (2015). Optimising emulsion stability during processing of model infant formulae using factorial statistical design. *International Journal of Dairy Technology*, *68*(3), 334–341. https://doi.org/10.1111/1471-0307.12240

McCarthy, N. A., Kelly, A. L., O'Mahony, J. A., Hickey, D. K., Chaurin, V., & Fenelon, M. A. (2012). Effect of protein content on emulsion stability of a model infant formula. *International Dairy Journal*, *25*(2), 80–86. https://doi.org/10.1016/j.idairyj.2012.03.003

McClements, D. J. (2012). Crystals and crystallization in oil-in-water emulsions: Implications for emulsion-based delivery systems. *Advances in Colloid and Interface Science*, *174*, 1–30. https://doi.org/10.1016/j.cis.2012.03.002

McClements, D. J., & Gumus, C. E. (2016). Natural emulsifiers — Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Advances in Colloid and Interface Science*, *234*, 3–26. https://doi.org/10.1016/j.cis.2016.03.002

McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science*, *159*, 213–228. https://doi.org/10.1016/j.cis.2010.06.010

McClements, D. J., & Mahdi Jafari, S. (2018). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science*, *251*, 55–79. https://doi.org/10.1016/j.cis.2017.12.001

Miller, R., Ferri, J. K., Javadi, A., Krägel, J., Mucic, N., & Wüstneck, R. (2010). Rheology of interfacial layers. *Colloid and Polymer Science*, *288*, 937–950. https://doi.org/10.1007/s00396-010-2227-5

Millqvist-Fureby, A. (2003). Characterisation of spray-dried emulsions with mixed fat phases. *Colloids and Surfaces B: Biointerfaces*, *31*(1–4), 65–79. https://doi.org/10.1016/S0927-7765(03)00044-4

Mitropoulos, V., Mütze, A., & Fischer, P. (2014). Mechanical properties of protein adsorption layers at the air/water and oil/water interface: A comparison in light of the thermodynamical stability of proteins. *Advances in Colloid and Interface Science*, *206*, 195–206. https://doi.org/10.1016/j.cis.2013.11.004

Moro, A., Báez, G. D., Ballerini, G. A., Busti, P. A., & Delorenzi, N. J. (2013). Emulsifying and foaming properties of β-lactoglobulin modified by heat treatment. *Food Research International*, *51*(1), 1–7. https://doi.org/10.1016/j.foodres.2012.11.011

Munoz-Ibanez, M., Azagoh, C., Dubey, B. N., Dumoulin, E., & Turchiuli, C. (2015). Changes in oil-in-water emulsion size distribution during the atomization step in spray-drying encapsulation. *Journal of Food Engineering*, *167*, 122–132. https://doi.org/10.1016/j.jfoodeng.2015.02.008

Murray, B. S., & Dickinson, E. (1996). Interfacial rheology and dynamic properties of adsorbed films of food proteins and surfactants. *Food Science and Technology International*, *2*(3), 131–145. https://doi.org/10.3136/fsti9596t9798.2.131

Mwangi, W. W., Lim, H. P., Low, L. E., Tey, B. T., & Chan, E. S. (2020). Food-grade Pickering emulsions for encapsulation and delivery of bioactives. *Trends in Food Science & Technology*, *100*, 320–322. https://doi.org/10.1016/j.tifs.2020.04.020

Neumann, S. M., van der Schaaf, U. S., & Karbstein, H. P. (2018). Investigation on the relationship between interfacial and single droplet experiments to describe instability mechanisms in double emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *553*, 464–471. https://doi.org/10.1016/j.colsurfa.2018.05.087

Nijdam, J. J., & Langrish, T. A. G. (2006). The effect of surface composition on the functional properties of milk powders. *Journal of Food Engineering*, *77*(4), 919–925. https://doi.org/10.1016/j.jfoodeng.2005.08.020

O'Sullivan, J. J., Norwood, E. A., O'Mahony, J. A., & Kelly, A. L. (2019). Atomisation technologies used in spray drying in the dairy industry: a review. *Journal of Food Engineering*, *243*, 57–69. https://doi.org/10.1016/j.jfoodeng.2018.08.027

Oberdisse, J., & Hellweg, T. (2017). Structure, interfacial film properties, and thermal fluctuations of microemulsions as seen by scattering experiments. *Advances in Colloid and Interface Science*, *247*, 354–362. https://doi.org/10.1016/j.cis.2017.07.011

Park, J. D., Myung, J. S., & Ahn, K. H. (2016). A review on particle dynamics simulation techniques for colloidal dispersions : Methods and applications. *Korean Journal of Chemical Engineering*, *33*(11), 3069–3078. https://doi.org/10.1007/s11814-016-0229-9

Pasquali, R. C., Sacco, N., & Bregni, C. (2009). The studies on hydrophilic-lipophilic balance (HLB): Sixty years after William C . Griffin's pioneer work (1949-2009). *Latin American Journal of Pharmacy*, *28*(2), 313–317.

Perez, A. A., Carrera Sánchez, C., Rodríguez Patino, J. M., Rubiolo, A. C., & Santiago, L. G. (2010). Milk whey proteins and xanthan gum interactions in solution and at the air-water interface: A rheokinetic study. *Colloids and Surfaces B: Biointerfaces*, *81*(1), 50–57. https://doi.org/10.1016/j.colsurfb.2010.06.021

Petrovic, L. B., Sovilj, V. J., Katona, J. M., & Milanovic, J. L. (2010). Influence of polymer-surfactant interactions on o/w emulsion properties and microcapsule formation. *Journal of Colloid and Interface Science*, *342*(2), 333–339. https://doi.org/10.1016/j.jcis.2009.10.077

Pichot, R., Watson, R. L., & Norton, I. T. (2013). Phospholipids at the interface: Current trends and challenges. *International Journal of Molecular Sciences*, *14*(6), 11767–11794. https://doi.org/10.3390/ijms140611767

Prasad Reddy, M. N., Padma Ishwarya, S., & Anandharamakrishnan, C. (2019). Nanoencapsulation of roasted coffee bean oil in whey protein wall system through nanspray drying. *Journal of Food Processing and Preservation*, *43*, 13893. https://doi.org/10.1111/jfpp.13893

Pugnaloni, L. A., Dickinson, E., Ettelaie, R., Macke, A. R., & Wilde, P. J. (2004). Competitive adsorption of proteins and low-molecular-weight surfactants: computer simulation and microscopic imaging. *Advances in Colloid and Interface Science*, *107*, 27–49. https://doi.org/10.1016/j.cis.2003.08.003

Radtke, T., Böni, L., Bohnacker, P., Maggi-beba, M., Fischer, P., Kriemler, S., Benden, C., & Dressel, H. (2018). Acute effects of combined exercise and oscillatory positive expiratory pressure therapy on sputum properties and lung diffusing capacity in cystic fibrosis : a randomized , controlled , crossover trial. *BMC Pulmonary Medicine*, *18*(99), 1–12. https://doi.org/10.1186/s12890-018-0661-1

Ramakrishnan, S., Ferrando, M., Aceña-Muñoz, L., Mestres, M., De Lamo-Castellví, S., & Güell, C. (2014). Influence of emulsification technique and wall composition on physicochemical properties and oxidative stability of fish oil microcapsules produced by spray drying. *Food and Bioprocess Technology*, *7*(7), 1959–1972. https://doi.org/10.1007/s11947-013-1187-4

Ravera, F., Dziza, K., Santini, E., Cristofolini, L., & Liggieri, L. (2020). Emulsification and emulsion stability: the role of the interfacial properties. *Advances in Colloid and Interface Science*, *288*, 102344. https://doi.org/10.1016/j.cis.2020.102344

Ravera, F., Loglio, G., & Kovalchuk, V. I. (2010). Interfacial dilational rheology by oscillating bubble/drop methods. *Current Opinion in Colloid and Interface Science*, *15*(4), 217–228. https://doi.org/10.1016/j.cocis.2010.04.001

Renggli, D., Alicke, A., Ewoldt, R. H., & Vermant, J. (2020). Operating windows for oscillatory interfacial shear rheology. *Journal of Rheology*, *64*(1), 141–160. https://doi.org/10.1122/1.5130620

Ribeiro, A. P. B., Masuchi, M. H., Miyasaki, E. K., Domingues, M. A. F., Stroppa, V. L. Z., de Oliveira, G. M., & Kieckbusch, T. G. (2015). Crystallization modifiers in lipid systems. *Journal of Food Science and Technology*, *52*(7), 3925–3946. https://doi.org/10.1007/s13197-014-1587-0

Rodríguez Patino, J. M., Navarro García, J. M., & Rodríguez Nio, M. R. (2001). Protein-lipid interactions at the oil-water interface. *Colloids and Surfaces B: Biointerfaces*, *21*(1–3), 207–216. https://doi.org/10.1016/S0927-7765(01)00173-4

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein-polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*, 1925–1937. https://doi.org/10.1016/j.foodhyd.2011.02.023

Rodríguez Patino, J. M., Rodríguez Nino, M. R., Carrera Sánchez, C., Navarro García, J. M., Rodríguez Mateo, G. R., & Cejudo Fernández, M. (2001). The effect of temperature on food emulsifiers at fluid-fluid interfaces. *Colloids and Surfaces B: Biointerfaces*, *21*(1–3), 87–99. https://doi.org/10.1016/S0927-7765(01)00187-4

Rodríguez Patino, J. M., Rosario Rodríguez Nino, M., & Carrera Sánchez, C. (2007). Physico-chemical properties of surfactant and protein films. *Current Opinion in Colloid and Interface Science*, *12*, 187–195. https://doi.org/10.1016/j.cocis.2007.06.003

Roos, Y. H. (2002). Importance of glass transition and water activity to spray drying and stability of dairy powders. *Lait*, *82*, 475–484. https://doi.org/10.1051/lait:2002025

Roos, Y. H. (2010). Glass transition temperature and its relevance in food processing. *Annual Review of Food Science and Technology*, *1*(1), 469–496. https://doi.org/10.1146/annurev.food.102308.124139

Roos, Y. H., & Drusch, S. (Eds). (2016). *Phase transitions in foods* (Second edi). Academic Press, Oxford, UK.

Roos, Y. H., & Karel, M. (1991). Phase transitions of mixtures of amorphous polysaccharides and sugars. *Biotechnology Progresslogy*, *7*, 49–53. https://doi.org/10.1021/bp00007a008

Roth, S., Murray, B. S., & Dickinson, E. (2000). Interfacial shear rheology of aged and heat-treated beta-lactoglobulin films: Displacement by nonionic surfactant. *Journal of Agricultural and Food Chemistry*, *48*, 1491–1497. https://doi.org/10.1021/jf990976z

Rühs, P. A., Affolter, C., Windhab, E. J., & Fischer, P. (2013). Shear and dilatational linear and nonlinear subphase controlled interfacial rheology of β-lactoglobulin fibrils and their derivatives. *Journal of Rheology*, *57*, 1003–1022. https://doi.org/10.1122/1.4802051

Rühs, P. A., Scheuble, N., Windhab, E. J., Mezzenga, R., & Fischer, P. (2012). Simultaneous control of ph and ionic strength during interfacial rheology of β-lactoglobulin fibrils adsorbed at liquid/liquid interfaces. *Langmuir*, *28*(34), 12536–12543. https://doi.org/10.1021/la3026705

Ruíz-Henestrosa, V. P., Sánchez, C. C., & Rodríguez Patino, J. M. (2008). Effect of sucrose on functional properties of soy globulins: Adsorption and foam characteristics. *Journal of Agricultural and Food Chemistry*, *56*(7), 2512–2521. https://doi.org/10.1021/jf0731245

Sagis, L. M. C., & Fischer, P. (2014). Nonlinear rheology of complex fluid-fluid interfaces. *Current Opinion in Colloid and Interface Science*, *19*(6), 520–529. https://doi.org/10.1016/j.cocis.2014.09.003

Sanchez-Reinoso, Z., & Gutiérrez, L. F. (2017). Effects of the emulsion composition on the physical properties and oxidative stability of Sacha Inchi (Plukenetia volubilis L.) oil microcapsules produced by spray drying. *Food and Bioprocess Technology*, *10*(7), 1354–1366. https://doi.org/10.1007/s11947-017-1906-3

Sánchez, C. C., & Rodríguez Patino, J. M. (2004). Flow-induced molecular segregation in β-casein-monoglyceride mixed films spread at the air-water interface. *Langmuir*, *20*(15), 6327–6334. https://doi.org/10.1021/la049625r

Santana, R. C., Perrechil, F. A., & Cunha, R. L. (2013). High- and low-energy emulsifications for food applications: A focus on process parameters. *Food Engineering Reviews*, *5*(2), 107–122. https://doi.org/10.1007/s12393-013-9065-4

Santos, D., Maurício, A. C., Sencadas, V., Santos, J. D., Fernandes, M. H., & Gomes, P. S. (2018). Spray drying: An overview. *Biomaterials - Physics and Chemistry - New Edition*. https://doi.org/10.5772/intechopen.72247

Schestkowa, H., Drusch, S., & Wagemans, A. M. (2020). FTIR analysis of β-lactoglobulin at the oil/water-interface. *Food Chemistry*, *302*, 125349. https://doi.org/10.1016/j.foodchem.2019.125349

Schestkowa, H., Wollborn, T., Westphal, A., Wagemans, A. M., Fritsching, U., & Drusch, S. (2019). Conformational state and charge determine the interfacial stabilization process of beta-lactoglobulin at preoccupied interfaces. *Journal of Colloid and Interface Science*, *536*, 300–309. https://doi.org/10.1016/j.jcis.2018.10.043

Schmitz, I., Gianfrancesco, A., Kulozik, U., & Foerst, P. (2011). Influence of temperature and the physical state on available lysine in powdered infant formula. *Procedia Food Science*, *1*, 1031–1038. https://doi.org/10.1016/j.profoo.2011.09.154

Schutyser, M. A. I., Perdana, J., & Boom, R. M. (2012). Single droplet drying for optimal spray drying of enzymes and probiotics. *Trends in Food Science and Technology*, *27*(2), 73–82. https://doi.org/10.1016/j.tifs.2012.05.006

Semenova, M. G. (2007). Thermodynamic analysis of the impact of molecular interactions on the functionality of food biopolymers in solution and in colloidal systems. *Food Hydrocolloids*, *21*(1), 23–45. https://doi.org/10.1016/j.foodhyd.2006.02.009

Semenova, M. G., & Dickinson, E. (2010). *Biopolymers in food colloids: Thermodynamics and molecular interactions* (E. Burlakova & G. Zaikov (eds.)). Koninklijke Brill NV, Leiden, The Netherlands. https://doi.org/10.1201/b12817

Serfert, Y., Schröder, J., Mescher, A., Laackmann, J., Rätzke, K., Shaikh, M. Q., Gaukel, V., Moritz, H. U., Schuchmann, H. P., Walzel, P., Drusch, S., & Schwarz, K. (2013). Spray drying behaviour and functionality of emulsions with β-lactoglobulin/pectin interfacial complexes. *Food Hydrocolloids*, *31*(2), 438–445. https://doi.org/10.1016/j.foodhyd.2012.11.037

Shukla, D., Schneider, C. P., & Trout, B. L. (2011). Molecular level insight into intra-solvent interaction effects on protein stability and aggregation. *Advanced Drug Delivery Reviews*, *63*(13), 1074–1085. https://doi.org/10.1016/j.addr.2011.06.014

Sprick, B., Linghu, Z., Amamcharla, J. K., Metzger, L. E., & Smith, J. S. (2019). Selective extraction of phospholipids from whey protein phospholipid concentrate using supercritical carbon dioxide and ethanol as a co-solvent. *Journal of Dairy Science*, *102*(12), 10855–10866. https://doi.org/10.3168/jds.2019-16419

Stefaniu, C., & Brezesinski, G. (2014). Grazing incidence X-ray diffraction studies of condensed double-chain phospholipid monolayers formed at the soft air/water interface. *Advances in Colloid and Interface Science*, *207*, 265–279. https://doi.org/10.1016/j.cis.2014.01.005

Taboada, M. L., Karbstein, H. P., & Gaukel, V. (2019). Spray drying of oil-in-water emulsions : oil droplet breakup during the atomization by pressure-swirl atomizers. *Proceedings of the 29th European Conference Liquid Atomization & Spray Systems, Paris*, *September*, 2–4.

Taboada, M. L., Leister, N., Karbstein, H. P., & Gaukel, V. (2020). Influence of the emulsifier system on breakup and coalescence of oil droplets during atomization of oil-in-water emulsions. *ChemEngineering*, *4*(3), 47. https://doi.org/10.3390/chemengineering4030047

Tajuelo, J., & Rubio, M. A. (2018). Flow field based data processing for the oscillating conical bob interfacial shear rheometer. *Journal of Rheology*, *62*, 295–311. https://doi.org/10.1122/1.5012764

Talón, E., Lampi, A., Vargas, M., Chiralt, A., Jouppila, K., & Gonzalez-Martinez, C. (2019). Encapsulation of eugenol by spray-drying using whey protein isolate or lecithin : release kinetics, antioxidant and antimicrobial properties. *Food Chemistry*, *295*, 588–598. https://doi.org/10.1016/j.foodchem.2019.05.115

Tamm, F., Sauer, G., Scampicchio, M., & Drusch, S. (2012). Pendant drop tensiometry for the evaluation of the foaming properties of milk-derived proteins. *Food Hydrocolloids*, *27*(2), 371–377. https://doi.org/10.1016/j.foodhyd.2011.10.013

Taneja, A., Ye, A., Jones, J. R., Archer, R., & Singh, H. (2013). Behaviour of oil droplets during spray drying of milk-protein-stabilised oil-in-water emulsions. *International Dairy Journal*, *28*, 15–23. https://doi.org/10.1016/j.idairyj.2012.08.004

Tavares, G. M., Croguennec, T., Carvalho, A. F., & Bouhallab, S. (2014). Milk proteins as encapsulation devices and delivery vehicles: Applications and trends. *Trends in Food Science & Technology*, *37*(1), 5–20. https://doi.org/10.1016/j.tifs.2014.02.008

Tippetts, M., & Martini, S. (2009). Effect of cooling rate on lipid crystallization in oil-in-water emulsions. *Food Research International*, *42*, 847–855. https://doi.org/10.1016/j.foodres.2009.03.009

Troise, A. D., & Fogliano, V. (2013). Reactants encapsulation and Maillard reaction. *Trends in Food Science & Technology*, *33*, 63–74. https://doi.org/10.1016/j.tifs.2013.07.002

Ulbrich, M., Daler, J. M., & Flöter, E. (2019). Acid hydrolysis of corn starch genotypes. I. Impact on morphological and molecular properties. *Carbohydrate Polymers*, *219*, 172–180. https://doi.org/10.1016/j.carbpol.2019.05.010

Ulbrich, M., Terstegen, T. A., & Flöter, E. (2019). Molecular investigation of the gel structure of native starches. *Starch/Staerke*, *71*, 1–9. https://doi.org/10.1002/star.201800080

Van Nieuwenhuyzen, W., & Tomás, M. C. (2008). Update on vegetable lecithin and phospholipid technologies. *European Journal of Lipid Science and Technology*, *110*(5), 472–486. https://doi.org/10.1002/ejlt.200800041

Vega, C., & Roos, Y. H. (2006). Invited review: spray-dried dairy and dairy-like emulsions - compositional considerations. *Journal of Dairy Science*, *89*, 383–401. https://doi.org/10.3168/jds.S0022-0302(06)72103-8

Vignolles, M.-L., Jeantet, R., Lopez, C., & Schuck, P. (2007). Free fat, surface fat and dairy powders: interactions between process and product. A review. *Lait*, *87*, 187–236. https://doi.org/10.1051/lait:2007010

Vollhardt, D., & Brezesinski, G. (2015). Monolayer characteristics of 1-monostearoyl-rac-glycerol at the air-water interface. *Journal of Physical Chemistry C*, *119*(18), 9934–9946. https://doi.org/10.1021/acs.jpcc.5b01766

Walstra, P. (2003). *Physical Chemistry of Foods*. Marcel Dekker, Inc. https://doi.org/10.1201/9780203910436

Walstra, P., Wouters, J. T. M., & Geurts, T. J. (2006). *Concentration processes, milk liquid consumption, milk powder and milk components in Dairy Science and Technology* (Vol. 4). Taylor & Francis Group, LLC. isbn:9780824727635

Wang, Shujie, Shi, Y., Tu, Z., Zhang, L., Wang, H., Tian, M., & Zhang, N. (2017). Influence of soy lecithin concentration on the physical properties of whey protein isolate-stabilized emulsion and microcapsule formation. *Journal of Food Engineering*, *207*, 73–80. https://doi.org/10.1016/j.jfoodeng.2017.03.020

Wang, Shujun, Li, C., Copeland, L., Niu, Q., & Wang, S. (2015). Starch retrogradation: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *14*(5), 568–585. https://doi.org/10.1111/1541-4337.12143

Ward, A. F. H., & Tordai, L. (1946). Time-dependence of boundary tensions of solutions I. The role of diffusion in time-effects. *The Journal of Chemical Physics*, *14*(1946), 453–461. https://doi.org/10.1063/1.1724167

Westergaard, V. (2004). *Milk powder technology: evaporation and spray drying*. https://www.gea.com/en/binaries/Milk Powder Technology - Evaporation and Spray Drying_tcm11-33784.pdf

Whitehurst, R. J. (Eds). (2004). *Emulsifiers in food technology*. Blackwell Publishing Ltd, Oxford, UK.

Wilde, P., Mackie, A., Husband, F., Gunning, P., & Morris, V. (2004). Proteins and emulsifiers at liquid interfaces. *Advances in Colloid and Interface Science*, *108–109*, 63–71. https://doi.org/10.1016/j.cis.2003.10.011

Wüstneck, R., Moser, B., & Muschiolik, G. (1999). Interfacial dilational behaviour of adsorbed β-lactoglobulin layers at the different fluid interfaces. *Colloids and Surfaces B: Biointerfaces*, *15*, 263–273. https://doi.org/10.1016/S0927-7765(99)00093-4

Yampolskaya, G., & Platikanov, D. (2006). Proteins at fluid interfaces: Adsorption layers and thin liquid films. *Advances in Colloid and Interface Science*, *128–130*(2006), 159–183. https://doi.org/10.1016/j.cis.2006.11.018

Yan, X., Ma, C., Cui, F., McClements, D. J., Liu, X., & Liu, F. (2020). Protein-stabilized Pickering emulsions: Formation, stability, properties, and applications in foods. *Trends in Food Science & Technology*, *103*, 293–303. https://doi.org/10.1016/j.tifs.2020.07.005

Yesiltas, B., Torkkeli, M., Almásy, L., Dudás, Z., Wacha, A. F., Dalgliesh, R., García-Moreno, P. J., Sørensen, A. D. M., Jacobsen, C., & Knaapila, M. (2019). Interfacial structure of 70% fish oil-in-water emulsions stabilized with combinations of sodium caseinate and phosphatidylcholine. *Journal of Colloid and Interface Science*, *554*, 183–190. https://doi.org/10.1016/j.jcis.2019.06.103

Zafar, U., Vivacqua, V., Calvert, G., Ghadiri, M., & Cleaver, J. A. S. (2017). A review of bulk powder caking. *Powder Technology*, *313*, 389–401. https://doi.org/10.1016/j.powtec.2017.02.024

Zhai, J., Miles, A. J., Pattenden, L. K., Lee, T.-H., Augustin, M. A., Wallace, B. A., Aguilar, M.-I., & Wooster, T. J. (2010). Changes in beta-lactoglobulin conformation at the oil/water interface of emulsions studied by synchrotron radiation circular dichroism spectroscopy. *Biomacromolecules*, *11*(8), 2136–2142. https://doi.org/10.1021/bm100510j

Zhou, B., Tobin, J. T., Drusch, S., & Hogan, S. A. (2020). Interfacial properties of milk proteins: a review. *Advances in Colloid and Interface Science*, *102347*. https://doi.org/10.1016/j.cis.2020.102347

Ziaee, A., Albadarin, A. B., Padrela, L., Femmer, T., O'Reilly, E., & Walker, G. (2019). Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches. *European Journal of Pharmaceutical Sciences*, *127*, 300–318. https://doi.org/10.1016/j.ejps.2018.10.026

Zou, L., & Akoh, C. C. (2013). Characterisation and optimisation of physical and oxidative stability of structured lipid-based infant formula emulsion: effects of emulsifiers and biopolymer thickeners. *Food Chemistry*, *141*(3), 2486–2494. https://doi.org/10.1016/j.foodchem.2013.05.029

ANNEX – Author details

Publications

- **Manuscript 1:**
  Heiden-Hecht, T., Taboada, M. L., Brückner-Gühmann, M., Karbstein, H. P., Gaukel, V. and Drusch, S. (2021). Towards an improved understanding of spray-dried emulsions: impact of the emulsifying constituent combination on characteristics and storage stability. International dairy journal, 105134. doi: 10.1016/j.idairyj.2021.105134
- **Manuscript 2:**
  Taboada, M. L., Heiden-Hecht, T., Brückner-Gühmann, M., Karbstein, H. P., Drusch, S. and Gaukel, V. (2021). Spray drying of emulsions: influence of the emulsifier system on changes in oil droplet size during the drying step. Journal of Food Processing and Perservation, 45, e15753. doi: 10.1111/jfpp.15753
- **Manuscript 3:**
  Heiden-Hecht, T., Ulbrich, M., Drusch, S., Brückner-Gühmann, M. (2021). Interfacial properties of β-lactoglobulin at the oil/water interface: influence of starch conversion products with varying dextrose equivalents. Food Biophysics, 16, 169-180. doi: 10.1007/s11483-020-09658-4
- **Manuscript 4:**
  Heiden-Hecht, T. and Drusch, S. (2021). Impact of saturation of fatty acids of phospholipids and oil phase on properties of β-lactoglobulin at the oil/water interface. Food Biophysics, accepted.

- Brückner-Gühmann, M., Heiden-Hecht, T., Sözer, N., Drusch, S. (2018). Foaming characteristics of oat protein and modification by partial hydrolysis. European food research and technology 244, 2095-2106. doi: 10.1007/s00217-018-3118-0
- Einhorn-Stoll, U., Kastner, H., Hecht, T., Zimathies, A., Drusch, S. (2015). Modification and physico-chemical properties of citrus pectin - Influence of enzymatic and acidic demethoxylation. Food Hydrocolloids 51, 338-345. doi: 10.1016/j.foodhyd.2015.05.031

Conference contributions

- Heiden-Hecht, T., Brückner-Gühmann, M., Drusch, S. (2021). Zusammenhang der Stabilität sprühgetrockneter Emulsionen und der formulierungsabhängigen Grenzflächeneigenschaften von β-lactoglobulin. ProcessNet-Fachgruppen Lebensmittelverfahrens-technik, Misch-vorgänge, Grenzflächenbestimmte Systeme und Prozesse. Online.
- Brückner-Gühmann, M., Heiden-Hecht, T., Kratsch, A., Drusch, S. (2020). Enzymatic modification of oat protein – tailoring of functional properties. NIZO Plant Protein Conference. Online.
- Heiden-Hecht, T., Brückner-Gühmann, M., Drusch, S. (2019). Towards an improved stability of spray-dried emulsions: impact of formulation on interfacial properties of β-lactoglobulin. 11. NIZO Dairy Conference in Pappendal, Netherlands.
- Hecht, T., Brückner-Gühmann, M., Drusch, S. (2016). Das Potential von Haferproteinisolat und -hydrolysat in Lebensmittelschäumen. GDL – Konferenz in Lemgo, Germany.
- Einhorn-Stoll, U., Vasileva, E., Hecht, T., Drusch, S. (2015). Investigation of pectin-water interactions - A practical approach. In Peter Williams and Glyn Phillips, Gums and Stabilizers for the Food Industry Conference 18, Wrexham, GB.

Awards

- **J.T.M. Wouters Young Scientist Award,** 3rd place NIZO Conference (10/2019)
- **VDI price** for excellent master's degree in engineering (10/2017)