

Strategies to Increase Turbidity in Beverage Emulsions

vorgelegt von
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von der Fakultät III – Prozesswissenschaften
der Technischen Universität Berlin
zur Erlangung des akademischen Grades

Doktorin der Ingenieurwissenschaften
- Dr.-Ing -

genehmigte Dissertation

Promotionsausschuss

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Tag der wissenschaftlichen Aussprache: 11. Oktober 2017

Berlin 2018

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von

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Acknowledgements

First and foremost, I would like to thank Prof. Dr. Stephan Drusch to take on this journey with me unseen. Without his ongoing guidance and support this thesis would not have been possible. I highly appreciate the valuable scientific advice and the constant interest in the progress of my thesis.

Equally important in realizing this project is the support from Dr. Gerhard Krammer, Senior Vice President Research & Technology Symrise Flavor Division. I am sincerely grateful for the opportunity to conduct this research project!

This work would not have been possible without the help of Dr. Christopher Sabater, Director Technology Research. Thank you very much for helping and supporting me and for your interest in this project.

I want to express my heartfelt gratitude to Dr. Jenny Weißbrodt, Director Technology Scouting and Development, for her guidance and supervision. I highly appreciate the support and the discussions throughout all phases and by all measures (including sugar and alcohol in cases of emergency). Your door was always open for me.

I further owe a debt of gratitude to the beverage crew, Jens Fahle, Dieter Kumlehn and especially Dr. Katja Obst, “hat” of beverages, for adopting me and giving me shelter in an air-conditioned lab, for endless beverage supply and most of all for teaching me in the art of creating beverage emulsions.

My cordial thanks go to Prof. Dr. Gisela Guthausen, Institute of Mechanical Process Engineering and Mechanics, for my wonderful stay at Karlsruhe Institute of Technology. I highly appreciate our fruitful collaboration and her incredible support developing a TD-NMR measurement with me.

My special thanks go to all the dedicated people who tried to analyze my samples by high resolution NMR: Andreas Kretzer and Dr. Reiner Dieden at Symrise as well as Dr. Sebastian Kemper and Dr. Jan Dirk Epping from TU Berlin for following my crazy ideas and answering patiently the questions I bombarded them with.

I would also like to thank my students, Maria Schwiipp and Svenja Wojciekowski, for their hard work supporting my research and Jan Flormann, the best apprentice ever.

I highly appreciate the helpfulness and support of Olga Pauli, Irene Frank, Frank Aickele and Alexander Diring in the everyday laboratory routine and the fun we had along. I also want to thank my first level graphics support, Ingo Lindner.

Special thanks go to Frederic Tamm and Janine Toppel for introducing me to interfacial rheology in the heat of the Berlin summer. My thanks also go to Adrian Kape, Friedrike Gutöhrlein, Anja Oechsele, Christine Ludewig and all my colleagues at the Department of Food Technology and Material Science at the TU Berlin for instant support at my short visits.

Katrin, your door was always open for me. I am deeply thankful for countless sleep-overs, heated discussions about the scattering of light and standing by my side after rough days.

Last but not least I would like to thank my family and close friends who supported me in in so many ways. Especially Mo, you are the best! From day one you have been my biggest fan, always believing in me. Without you I would have never picked up on this challenge.

Zusammenfassung

Carbonisierte Erfrischungsgetränke enthalten für ein trübes Erscheinungsbild häufig Getränkeemulsionen. Diese sogenannten „Clouds“ sollen die Natürlichkeit fruchtbasierter Getränke unterstreichen. Ziel der vorliegenden Arbeit war es, Strategien zu entwickeln, die Trübungskraft von Getränkeemulsionen zu erhöhen. Um dies zu erreichen, musste zunächst eine Korrelation zwischen der optischen Wahrnehmung des Menschen und den Messwerten der Trübungsmessung hergestellt werden. Es wurde ein polynominaler Zusammenhang zwischen der menschlichen Wahrnehmung und den Trübungsmesswerten nachgewiesen. Außerdem wurden verschiedene sensible Bereiche festgestellt. Aus einer systematischen Untersuchung der Einflussfaktoren auf die Trübung ließ sich ableiten, dass neben der Öltropfengröße und -konzentration, die Brechungsindexdifferenz (BID) zwischen den beiden Phasen einer Emulsion signifikante Trübungsunterschiede erzeugt. Folglich basierten die Strategien zur Trübungserhöhung auf der Erhöhung der BID zwischen kontinuierlicher und disperser Phase, was mittels Modifizierung der Grenzfläche oder der dispersen Phase erfolgen sollte. Es wurde angenommen, dass sich die Lichtbrechung an der Grenzfläche verstärkt, wenn Partikel statt Emulgatoren zur Stabilisierung der Emulsion verwendet werden. Eine ausführliche Literaturrezension ergab, dass sich sogenannte Pickering-Partikel dafür eignen. Nichtsdestotrotz kann die notwendige physikalische Stabilität, die Getränkeemulsionen mit sich bringen müssen, nicht mit Pickering-Partikeln hergestellt werden. Die herausragende Stabilität von Pickering-Emulsionen basiert auf einem 3D-Netzwerk, welches bei der Verdünnung in die Endanwendung zwangsläufig zerstört wird. Alternativ wurden mittels eines Heißemulgiervorgangs hochschmelzende Emulgatoren verwendet. Die während des Abkühlens einsetzende Kristallisation des Emulgators sollte die lichtstreuenden Eigenschaften der Emulsion erhöhen. Die Ergebnisse ließen darauf schließen, dass die getesteten Emulgatoren nicht in ausreichendem Maße kristallisierten oder die Anordnung an der Grenzfläche die Ausbildung einer Kristallstruktur verhindert haben. Im Gegensatz dazu war der Austausch der dispersen Phase mit einem hochschmelzenden Fett erfolgreich und führte zu einer signifikanten Erhöhung der BID um 0,05–0,08 und 50 % mehr Trübung. Um überprüfen zu können, ob der Trübungsanstieg mit der Kristallinität der dispersen Phase zusammen hängt, musste der Festfettgehalt (FFG) bestimmt werden. Die klassische FFG-Bestimmung mittels Niederfeld-Magnetresonanz (TD-NMR) konnte

in diesem Fall nicht angewendet werden, da die Methode nur bis zu einem Wassergehalt von 15 % zuverlässig ist. Durch die Kombination von Inversion Recovery und T_2 -Relaxations-Experimenten – verbunden über ein probenspezifisches Inversion Delay – konnte eine Methode entwickelt werden, die es ermöglicht, in stark wasserhaltigen Proben flüssige und feste Fettanteile zu messen. Basierend auf den Ergebnissen konnte festgestellt werden, dass ein minimaler $\text{FFG}_{\text{krit}} > 36 \%$ notwendig ist, um signifikante Trübungsunterschiede zu erzeugen. Die Ergebnisse der vorliegenden Arbeit demonstrieren, dass eine Erhöhung der BID eine erfolgreiche Strategie darstellt, die Trübung in Getränkeemulsionen signifikant zu erhöhen, und die Verwendung von festen Fetten verursacht bei ausreichender Kristallisation eine höhere Lichtbrechung in Emulsionen.

Abstract

Carbonated soft drinks (CSD) often contain beverage emulsions to provide turbidity. These so-called clouds enhance the natural appearance of fruit-derived soft drinks. The aim of the present study was to develop a strategy to increase turbidity in beverage clouds. To fulfill this aim it was first necessary to understand the relation between human visual perception and instrumental turbidity measurement. A polynomial relationship between turbidity and the human perception was established. Depending on the intensity of the turbidity different ranges of sensitivity were found. In a systematic approach factors affecting turbidity were compared. It could be concluded that apart from droplet size and concentration the refractive index difference (RID) between the two phases of the emulsion accounts for significant changes in turbidity. Therefore strategies to increase turbidity were based on the RID between continuous and dispersed phase, which may be achieved either via modification of the interface or the dispersed phase itself. Scattering of light at the interface was expected to increase when particles are used for stabilization of an emulsion. Based on an extensive review RID may be modified via Pickering particles. However, it was concluded that physical stability in Pickering emulsion as a prerequisite for beverage emulsions is not provided. Superior stability of Pickering emulsions is based on 3D network formation which is destabilized upon dilution when preparing beverages with near water viscosity. As an alternative, high melting emulsifiers (HME) were investigated performing a hot homogenization procedure. Crystallization of the emulsifier upon cooling was expected to increase the scattering efficiency at the interface. The results indicated that crystallization of the tested emulsifiers did not occur to a sufficient degree or conformational changes at the interface inhibit the formation of a crystal network. In contrast, modification of the dispersed phase with high melting lipids (HML) resulted in a significant increase of the RID by 0.05-0.08 and a significant increase in turbidity of 50 %. To prove that this effect must be attributed to the crystallinity of the dispersed phase, the solid fat content (SFC) in dispersion had to be determined. Classical SFC measurement by TD-NMR is not suitable in the present study, since it only allows maximum water content of 15 %. A new procedure was established by combining an inversion recovery and T_2 -relaxation with a customized inversion delay and dedicated data processing. Based on this method it was shown that a minimum $SFC_{crit} > 36\%$ is required for significantly more turbid samples. Therefore, results of the present study clearly demonstrate that an increase in the RID of an

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List of Abbreviations

CE	coarse emulsion
CEB	coarse emulsion prepared with beverage base
CSD	carbonated soft drink
DSC	differential scanning calorimetry
EG	glycerol ester of wood rosin
FE	fine emulsion
FEB	fine emulsion prepared with beverage base
FID	free induction decay
GA	gum arabic
GSC	glyceryl stearoyl citrate
HLB	hydrophilic-lipophilic balance
HME	high melting emulsifiers
HML	high melting lipids
HPF	hydrogenated palm fat
HPK	hydrogenated palm kernel fat
ID	inversion delay
LSD	least significant difference
MCT	medium chain triglyceride
MCTW	weighted MCT oil
MS	modified starch
NMR	nuclear magnetic resonance
NTU	nephelometric turbidity units
OO	orange oil
OOW	weighted orange oil
OSA	octenyl-succinate derivatised starch
o/w	oil in water

PE	pre-emulsion
PEB	pre-emulsion prepared with beverage base
PL	phospholipon
PPK	palm kernel fat
Q	<i>Quillaja saponaria Molina</i> extract
RGD	Rayleigh-Gans-Debye
RI	refractive index
RID	refractive index difference
SFC	solid fat content
SOE	sucrose oligoester
SLP	solid lipid particle
SSL	sodium stearyl lactylate
SSS	tristearin
STD	saturation transfer differences
TAG	triacylglycerol
TD-NMR	time-domain nuclear magnetic resonance
TRDD	transverse relaxation decay deconvolution
WPC	whey protein concentrate

1. Introduction

Global soft drink consumption is roughly 600 billion liters per year as of 2015 (Karagiannis & Schäfer, 2016). This makes CSDs the most valuable soft drinks second only behind bottled water (Attias & Delpech, 2016). A CSD is either crystal clear like the common cream soda or turbid like most fruit-derived drinks. The latter are usually made from emulsions which provide water-insoluble flavors, and/or colors and/or turbidity to enhance the natural appearance of a juice containing beverage (Shachman, 2004, Piorkowski & McClements, 2014). Traditionally turbidity is provided through a flavoring system containing weighting agent. Due to legal constraints and the consumer demand for more natural products, new systems providing only turbidity have been developed. These so-called clouds consist of a neutral oil phase and a water phase with a food-grade emulsifier such as gum arabic (GA) or modified starch and preservatives. A typical dosage in the final application is 0.1 – 0.2 %. One problem associated with these systems is that the performance of the existing clouds does not meet customer expectations. As a consequence a more efficient approach for creating turbidity is required. Unfortunately, turbidity is not a physical property but rather the visual appearance, which makes it difficult to grasp (Thorne & Nannestad, 1959). Just recently Zhang & Reineccius (2016) concluded that the factors affecting turbidity are poorly understood.

According to DIN EN ISO 7027:2000-04, turbidity is the attenuation of light due to the presence of undissolved substances. If light traverses a perfectly homogenous medium, it is not scattered. The driving factor for the scattering of light is that light travels at different speeds in different media (Kao, 1995). As a result a beam of light is bent or refracted when entering a material with a different speed of light. This phenomenon can be expressed by the refractive index n of a medium, as given in Equation (1-1). The refractive index (RI) is defined as the ratio of the speed of light in vacuum, $c_0 = 299792458$ m/s, and the phase velocity c of light in the respective medium (Kuchling, 2004):

$$(1-1) \quad n = \frac{c_0}{c}$$

Looking deeper into the physics of the scattering of light of small particles, further factors influencing turbidity can be derived (Kao, 1995). The scattering of light is an ubiquitous natural

phenomenon inherent to emulsions (Kerker, 1969). The best known example is milk which does not only appear white because of the dispersed proteins but mainly because of the finely emulsified oil droplets. This actually happens in any dispersion because each dispersed droplet, even each molecule, acts as a scattering center. But it depends on the shape and the size of the molecules whether the scattering will be very effective (Van De Hulst, 1981). Since it can be assumed that the dispersed oil droplets in an emulsion will be spherical due to the least surface to volume ratio, the considerations of the scattering of spheres seems suitable. For very small particles, with particle sizes below the wavelength of the human visible spectrum, ($a < \lambda/20$), an isotropic scattering pattern occurs which is dependent on the wavelength of the light (see Figure 1-1a). It was first described by Lord Rayleigh (Rayleigh, 1881). For a small sphere with diameter d and refractive index n , where R is the distance to the particle and θ is the scattering angle, the intensity I of the scattered light is given by Equation (1-2):

$$(1-2) \quad I = \frac{I_0}{R^2} \frac{1 + \cos^2 \theta}{2} \left(\frac{2\pi}{\lambda} \right)^4 \left(\frac{n^2 - 1}{n^2 + 2} \right)^2 \left(\frac{d}{2} \right)^6$$

For infinitely small, non-absorbing particles in a non-absorbing medium Rayleigh's treatment is generally judged satisfactory, but if the particle size is nearly the same than the wavelength of light, or if absorption is present, the theoretical calculation of scattering becomes much more complex (Bailey et al., 1935). Subsequently the theory has been extended to the Rayleigh-Gans-Debye (RGD) approach to deal with larger particles. This RGD approach accounts for interference effects between light scattered from different parts of the same particle, or by an array of similar particles, and has been successfully applied to the study of polymers and colloids, yielding important information such as absolute molecular weight and particle size. The range of validity of the RGD approximation is given by Equation (1-3):

$$(1-3) \quad (2\pi D/\lambda) (m - 1) \ll 1$$

where D is the particle diameter and $m = n/n_0$ (n and n_0 being the refractive indices of the scatterer and the medium respectively). But it is compromising the phase shift, which starts and increases with increasing particle size. This means that the phase of the scattered wave differs, that is shifted, compared to the phase of the incident wave. Above the stated limit, the phase shift can no longer be neglected and for even bigger particles the mathematical treatment of the problem requires the solution of Maxwell's equations which was accomplished by Gustav Mie in

1908. As particles increase in size, the angular scattering pattern changes from the symmetric Rayleigh pattern to an anisotropic pattern characterized by an increased forward scattering (Figure 1-1b) (Becher, 1983). For even larger particles a structured pattern with a unique set of maxima and minima is detected as can be seen in Figure 1-1c. When illuminated with white light, the scattering appears white or milky compared to the blue Rayleigh scattering. For a given weight or volume of material the number of particles per unit volume of the system decreases inversely as the third power of the radius with increasing particle size. Meanwhile the scattering increases and the turbidity is the product of these quantities (Oster, 1948). When the particles are comparable in size to the wave length of incident light, the scattering per particle is proportional to the fourth power of the radius, and the scattering for a given weight or volume of material will increase linearly with the radius. For still bigger particles the scattering is proportional to the square of the radius, so that the scattering of the system will actually decrease with increasing radius of the particles. Thus, maximum scattering will occur for a given volume of material when

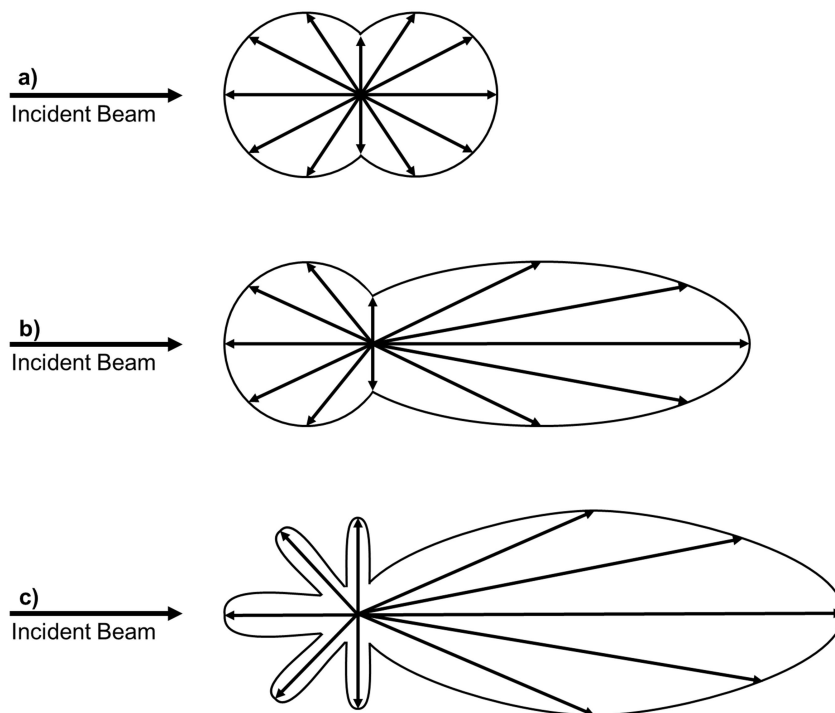


Figure 1-1: Size dependency on the scattering patterns of a) particles $< 0.45 \mu\text{m}$; b) particles $< 2 \mu\text{m}$; and c) particles $< 50 \mu\text{m}$ (adapted from Tilley, R. J. D. (2011). *Colour and the optical properties of materials: An exploration of the relationship between light, the optical properties of materials and colour* (2.ed). Chichester: Wiley.).

the particles are about equal in size to the wave length (Oster, 1948). Thus, Mie concluded from his calculations that the particle diameter for which the maximum of scattering is reached is proportional to the wave length (Mie, 1908). This description treats the system like a suspension of monodispersed hard spheres of refractive index n , dispersed in a homogeneous medium of refractive index n_0 . Other geometries than spheres are not strictly considered by the theory. However, the absolute magnitude of the scattering maxima and minima are extremely sensitive to polydispersity in size (Farinato & Rowell, 1983). In the case of an oil-in-water emulsion the major difficulty is the precise description of the interrelation between particles. The inter-particle effects can cause interference between scattered waves of neighbouring particles and is called dependent scattering (Drolen & Tien, 1987). This may not get confused with effects of multiple scattering. Multiple scattering is another phenomenon describing the effect when particles are exposed to light scattered by other particles, where the light of the original beam may have suffered extinction by the other particles. These effects can be strong and a simple proportionality does not exist (Van De Hulst, 1981). Kleizen concluded that for multiple scattering the amount of scattered light will be a higher-order function of the concentration. As multiple scattering depends on the length of the optical path, the accuracy of turbidity measurement can be increased by changing this length for one angle (Kleizen et al., 1995). Chantrapornchai et al. (1998) suggest, that the appearance of a concentrated emulsion is rather determined by the light which is reflected on the surface. For a concentrated water-in-oil emulsion it might not be possible to transmit a light beam because of the high degree of attenuation caused by droplet scattering.

Considering the complexity of the scattering of light, it is obvious that the appearance of a turbid oil-in-water emulsion is governed by a multitude of effects, like oil droplet size and concentration, polydispersity of oil droplet distribution and wave length of the incident light. It can be assumed that dependent and multiple scattering occurs as well as absorption for both phases.

Historically the measurement of turbidity was mainly undertaken to measure extremely low scattering. This is because devices have been developed to measure water clarity and for analytical applications like particle sizing or microbial growth, turbidity has to be measured in extreme dilution to guarantee single scattering. This explains why at the beginning most often photospectrometers, which measure transmittance (180 °), were used to detect absorption or scattering. But, for larger particles the forward scattering increases and interferes with the transmitted light resulting in lower readings or underestimated turbidity (Thorne, R. S. W., 1961).

With increasing interest in particle size characterization, research showed that especially for particles in the Mie range the detection angle of 90° to the incident beam proved to be highly effective and sensitive. The first nephelometers with 90° detection angle were established. With increasing interest of the measurement of high turbidity, for example in the beverage industry, new turbidimeters were developed. To be able to measure high turbid systems affected by broad scattering patterns, multiple scattering, absorption and reflection, up to four detection angles are installed around the probe and ratio values are calculated out of the different signals. But, no matter how advanced the measuring technique, the instrument needs to be calibrated with a turbidity standard as there is no such thing as a true turbidity (Thorne, 1961). This is why turbidity calibrated with formazine standards works well for measuring concentration of formazine solutions, but different systems may follow other concentration–turbidity relationships due to different particle size, shape or other optical properties. Thus, turbidimetric values are only a comparison to the scattering properties of the formazine standard. These values do not give any information about the human visual perception of beverage emulsions. Therefore, it is important to determine what differences in turbidity can be perceived by a human being and to establish a relationship to the instrumental readings of a typical beverage emulsion. Concluding, the human visual perception needs to be elucidated to be able to tailor the optical properties of beverage emulsions.

Furthermore, the aim of this study is to increase turbidity in oil-in-water beverage clouds. Therefore, it is interesting whether the emulsifier has an impact on the light scattering properties of the dispersed phase. Lately researchers found that the scattering pattern of nucleated particles, which have a buckeld surface, is broader than that of smooth surface particles (Chen et al., 2012) leading to the idea to modify the interface of an emulsion. Particles are able to stabilize emulsions, so-called Pickering emulsions. If the particles are neither soluble in water nor in oil they will accumulate at the interface providing a physical barrier to droplet coalescence (Tambe & Sharma, 1994b). Thus, suitable food-grade particles at the interface of beverage emulsions might enhance the scattering efficiency of the system. A continuation of the idea to use solids at the interface is to exchange the dispersed phase by solids in order to increase turbidity. The RI of a material increases when the state changes from liquid to solid. The solid i.e. crystalline form of a substance is the state of highest order. Since the phase relations in a crystal lattice are fixed, this leads to stronger external and internal interference of the light with the material compared to a

liquid (Giacovazzo, 2005). This physical phenomenon was e.g. observed by Helgason et al. (2015), who reported that spectrophotometric absorbance readings increased by 140 % upon crystallization of dispersed fat droplets in octadecane nanoemulsions. This demonstrates that solid fat could be used instead of liquid oil as dispersed phase.

It is known that the crystallization of fats in dispersion differs from the crystallization in bulk (Coupland, 2002; Douaire et al., 2014). This is due to the fact that a seed crystal would be needed in each oil droplet in order to start crystallization (McClements, 2012b). Therefore, determining the solid fat content (SFC) of the samples would be crucial to interpret the results. SFC is typically measured by Time-domain nuclear magnetic resonance (TD-NMR). NMR is based on the relaxation of magnetized protons after pulses of specific radio frequency. The classic, established SFC determination by TD-NMR relies on the fact that the liquid and solid parts of the oil phase can be differentiated because transverse relaxation is usually much faster for solids than for liquids. Standard methods are readily available for oil, spreads and seeds (DIN EN ISO 8292-1:2010-08:, 2010; DIN EN ISO 8292-2:2008:, 2010). However, conventional SFC determination with TD-NMR is only applicable for samples with a moisture content of 10 – 15 % (Todt et al., 2006). Samples with high amounts of free water cannot be treated this way. The NMR signal from water will interfere with the oil signal (Todt et al., 2006). Problems arise isolating the dispersed (droplet) phase signal from the quasi-continuous phase (Johns, 2009). In addition, the transverse water relaxation is strongly affected by the food network structure (Gianferri et al., 2007). A suitable method has to account for that and can be realized when combining longitudinal and transverse relaxation properties to separate the signal contribution of the quasi-continuous phase and the solid lipid and liquid lipid dispersed phase.

Concludingly, the aim of the present study, to understand the factors affecting turbidity in oil-in-water beverage clouds, was accomplished by investigating, the following aspects:

1. In psychophysics was shown for several stimuli that the human perception is not linear but can be described by a power law for example for sensations like loudness, warmth or cold (Zwislocki, 2009). Accordingly, it is hypothesized that the human visual perception of different intensities of turbidity does not follow the same linear correlation as the instrumental reading. On the other hand research and industry depend on fast and reliable measurement (Hongve & Åkesson, 1998) since sensory tests are time and labor intensive.

The correlation between the human visual perception and turbidity measurement is missing. Therefore, it is important to close that gap and the aim is to establish a relationship between the analytical and human response to turbidity (see Manuscript I in chapter 2). The results will be used as basis for the following research.

2. The factors influencing turbidity or rather the scattering of light is well described in physics for simple monodisperse systems (Kerker, 1969). Still, for the concentrated, polydisperse systems under investigation multiple scattering is expected. Kleizen et al. (1995) concluded that for multiple scattering the amount of scattered light will be a higher-order function of the concentration making predictions very complicated. For beverage emulsions it was just recently concluded that turbidity is poorly understood (Zhang & Reineccius, 2016). To modify the turbidity of a beverage emulsion the dimensions of the influence of the single factors need to be known. Therefore, it is important that the factors influencing turbidity in a specific model beverage system are empirically described in a systematic approach. It is hypothesized that the RID has the main impact on turbidity (Manuscript I, chapter 2).
3. Besides few exceptions, the solid i.e. crystalline form of a substance is the most densely packed structure of a material (Giacovazzo, 2005). Since every molecule adds to the scattering efficiency (Van De Hulst, 1981), the densest packing should induce the most scattering of light. The hypothesis is supported by Helgason et al. (2015) who observed increased absorption upon crystallization of dispersed lipid droplets. Consequently, one possibility would be to modify the interface of the emulsions with particles (Manuscript II, chapter 3) or other solid emulsifiers (Manuscript IV, chapter 5) to induce more scattering to the system. Another approach to increase the RID of the system is to exchange the liquid dispersed phase by a solid dispersed phase. This was done by using high melting lipids (HML) instead of liquid oils (Manuscript IV, chapter 5).
4. To be able to attribute an increase in turbidity to the crystallinity of the dispersed phase, the SFC in dispersion needs to be determined. TD-NMR is routinely used for SFC determination (DIN EN ISO 8292-1:2010-08, 2010; DIN EN ISO 8292-2:2008, 2010). But conventional methods are only applicable for samples with a moisture content of 10 – 15 % (Todt et al., 2006). Due to the high water content of beverage emulsions the determination of the SFC in dispersions with solid lipids is not readily available. NMR is

based on the relaxation of magnetized protons after pulses of specific radio frequency. It is hypothesized that by modifying a suitable pulse train program using TD-NMR the signal contribution of water can be eliminated (Manuscript III, chapter 4).

2. Manuscript I: Turbidity in oil-in-water-emulsions – key factors and visual perception

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Accepted manuscript in *Food Research International* 89 (2016) 202–210

<https://doi.org/10.1016/j.foodres.2016.07.019>

2.1. Abstract

The aim of the present study is to systematically describe the factors affecting turbidity in beverage emulsions and to get a better understanding of visual perception of turbidity. The sensory evaluation of the human visual perception of turbidity showed that humans are most sensitive to turbidity differences between two samples in the range between 1000 – 1500 NTU (ratio) (nephelometric turbidity units). At very high turbidity values more than 2000 TU in NTU (ratio) were needed to distinguish between samples that they were perceived significantly different. Particle size was the most important factor affecting turbidity. It was shown that a maximum turbidity occurs at a mean volume-surface diameter of 0.2 μm for the oil droplet size. Additional parameters were the refractive index, the composition of the aqueous phase and the presence of excess emulsifier. In a concentration typical for a beverage emulsion a change in the refractive index of the oil phase may allow the alteration of turbidity by up to 30 %. With the knowledge on visual perception of turbidity and the determining factors, turbidity can be tailored in product development according to the customer requirements and in quality control to define acceptable variations in optical appearance.

2.2. Introduction

The degree of turbidity is an important parameter reflecting the quality of a cloudy fruit juice (Kolniak-Ostek et al., 2013). Therefore the visual appearance of a cloudy drink is a critical factor for consumer acceptance (Mirhosseini & Tan, 2010). Turbidity in fruit-derived beverages gives a natural appearance, and is achieved through addition of so-called clouds or clouding agents to the majority of non-alcoholic beverages (Mirhosseini et al., 2008b); Taherian et al., 2006; Espachs-Barroso et al., 2005; Shachman, 2004).

A cloud provides turbidity, flavor, aroma, mouthfeel and optionally color and is typically added in concentrations of 0.01 – 0.2 wt % of the final product (Harnsilawat et al., 2006; Espachs-Barroso et al., 2005; Buffo et al., 2001; Tan, 1998; Dickinson, 1994). At this dosage turbidity usually ranges between 100 – 250 NTU (ratio). Clouds are concentrated oil-in-water emulsions in which the turbidity results from the scattering of light by dispersed oil droplets with oil droplet size between 0.5 – 5 μm (Tan, 1998; Dickinson, 1994). In this context the scattering of light is

governed by the size and concentration of the dispersed phase, the ratio of refractive index (RI) between dispersed and continuous phase and the wavelength and angle of the incident light (Kleizen et al., 1995). Physical background of the scattering of light has been reviewed extensively e.g. in Mie, 1908; Bailey et al., 1935; Oster, 1948; Lothian & Chappel, 1951; Walstra, 1965; Kerker, 1969; Van de Hulst, 1981; and Bohren & Huffman, 1983.

Concerning its composition a cloud is most commonly stabilized by amphiphilic polysaccharides, such as gum arabic or hydrophobically modified starch (Buffo et al., 2002; Channamai & McClements, 2001). The oil phase usually consists of vegetable oil, flavor oil, and a weighting agent (Tan, 1998). Weighting agents increase the density of the oil phase and therefore contribute to the stabilization of an emulsion through minimization of the difference in density between the different phases. A typical weighting agent is the glycerol ester of wood rosin (glyceryl abietate) (Lim et al., 2011; Given, 2009). With respect to the final application it needs to be mentioned that the aqueous phase of a beverage emulsions is normally acidic with a pH below 3 (Harnsilawat et al., 2006). A finished beverage typically contains additional sugar (10°Brix) and/or sweetener, citric acid and preservative (Reiner et al., 2010; Tan, 1998).

In the past turbidity measurement was mainly used as a tool to characterize clouding agents and cloud stability. The latter is the most critical quality parameter of a beverage emulsion is its stability in the diluted state (Kolniak-Ostek et al., 2013; Cao et al., 2012; Rao & McClements, 2012; Ibrahim et al., 2011; Reiner et al., 2010; Taherian et al., 2006; Harnsilawat et al., 2006; Liang et al., 2006; Dłużewska et al., 2004; Beveridge, 2002; Cameron et al., 1997; Sreenath et al., 1995; Morris, 1987; Kaufman & Garti, 1984; Ray et al., 1983; Herrera et al., 1979). In contrast, only little work has been done on the relationship between the human visual perception of turbidity and instrumental turbidity measurement. Few studies are available, in which minimum thresholds of the perception of turbidity were determined (Fleet & Siebert, 2005; 2006; Horne et al., 2001; Carrasco & Siebert, 1999; and Malcomson et al., 1989). Malcomson et al. (1989) examined the relationship of human perception of apple juice and instrumental turbidity values. He reported that a change in clarity was only observed by the sensory panel at a large difference in instrumental reading. This may be due to the fact, that turbidity was measured at a 90° detection angle which is very sensitive for small particles but which contribute only little to perceived turbidity. Another important factor might be the color of apple juice. Carrasco & Siebert (1999) found turbidity thresholds visible for the human eye below 1 NTU, but under very

controlled viewing conditions with a defined light source and a box with a black background. Horne et al. (2001) on the other hand measured visual haze detection thresholds for differently colored emulsions under less controlled conditions to simulate a consumer situation. The thresholds with up to 4.5 NTU reported are much higher than the results previously reported by the group of Carrasco & Siebert (1999), but overall are still very low. Furthermore, Carrasco & Siebert (1999) investigated the influence of color on the human visual perception of turbidity. The authors found that higher particle concentrations were needed to detect haze in red-colored suspensions compared with yellow-colored or clear suspensions. Fleet & Siebert (2005) compared in a similar study clear, caramel- and dark brown-colored suspensions and found thresholds between 0.2 – 2.2 NTU with the lowest values for clear and the highest results for dark brown-colored samples. As turbidity measurements were operated with white light, the readings were influenced by the color as well and might have produced more pronounced differences at NIR readings. The latter can be concluded from a study by Hongve & Åkesson (1998), who compared white light measurements against readings in the NIR region at 860 nm, and found that absorption due to color present reduces NTU (ratio) readings. On the other hand, at high turbidity levels saturation may occur and panelist are possibly not able any more to distinguish different levels of turbidity. Carrasco & Siebert (1999) concluded from a magnitude estimation study that the panelist response became saturated at higher turbidity values above 500 NTU. Anyhow no literature on the question, what difference in turbidity may be distinguished by human visual perception at a specific level of turbidity, is available. This holds particularly true for very high levels of turbidity up to 10.000 NTU as they may occur in cloudy beverages and juice concentrates.

From the literature outlined above it becomes evident that the correlation of human visual perception and instrumental reading is of major interest in consumer studies and the beverage industry. On the other hand studies have been performed to optimize beverage clouding systems (Gharibzahedi et al., 2012; Klein et al., 2010; Mirhosseini et al., 2009; (Mirhosseini et al., 2008b; Mirhosseini et al., 2008a; Mirhosseini et al., 2008c). Since these studies did not focus on turbidity, the factors affecting this parameter are still poorly understood. Therefore the aim of the present study was to systematically describe the factors affecting turbidity in beverage emulsions and to get a better understanding of consumer perception of turbidity. From an industrial perspective, with this data the development of a beverage can be more efficiently directed towards the desired

optical appearance. To fulfill this aim the impact of particle size, particle concentration, type of oil phase and type of emulsifier on turbidity were investigated for a polydisperse emulsion system. In addition, the instrumental turbidity measurement was correlated to the human visual perception via sensory analysis.

2.3 Materials and Methods

The following materials were purchased or kindly provided by the suppliers mentioned below: gum arabic Senegal type (GA, Nexira GmbH, München, Germany), medium chain triglyceride (MCT, Cremer Oleo GmbH & Co. KG, Hamburg, Germany), orange oil (OO, Citrosuco, Matão, Brazil), glycerol ester of wood rosin, beverage grade (EG, Pinova, Brunswick NJ, USA), α -tocopherol (BASF, Ludwigsburg, Germany), whey protein concentrate 80 % (WPC, FrieslandCampina DMV bv, Veghel, Netherlands), octenyl-succinate derivatized starch refined from waxy maize, *Quillaja saponaria Molina* extract (both National Starch & Chemical Ltd., Manchester, UK), potassium sorbate (Eastman Chemical Company, Kingsport, USA), citric acid (Jungbunzlauer Suisse AG, Basel, Switzerland), 72.7 °Brix inverted sugar syrup (Nordzucker AG, Braunschweig, Germany). To fulfill the aim of the study, different sets of experiments were performed.

2.3.1. Emulsion Preparation

A standard emulsion was prepared by dissolving gum arabic (13.5 % w/w) and preservatives in water with RW20DZM agitator with R 1302 dissolver stirrer (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and left over night for hydration. MCT (7.15 % w/w) was gradually added to the continuous phase while mixing with Ultra-Turrax T50 with dispersion unit S50N (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 6000 rpm for 5 minutes to generate a pre-emulsion (PE). The PE was left at least two hours for deaeration before homogenization with a laboratory table top model homogenizer NS1001L2K (GEA Niro Soavi S.p.A., Parma, Italy). Unless stated otherwise, homogenization was done in three cycles in a two stage process at 20/5 MPa. Concentrations for analytical purposes were made with deionized water. To estimate the error in multiple preparation in emulsions the standard emulsion was prepared in triplicate

resulting in a particle size of $\bar{x}_{1,2} = 0.3 \mu\text{m} \pm 0.01$ standard deviation and turbidity being $119.8 \text{ NTU (ratio)} \pm 6.92$ standard deviation.

2.3.2. Impact of particle size

To analyze the impact of particle size on turbidity emulsions with constant oil content but different particle size distributions had to be prepared. This was achieved by varying the homogenization pressure during emulsification of the standard emulsion. A first sample was taken after pre-emulsification. The PE was then divided into four equal portions, which were homogenized in three cycles at varying pressures (8/3 MPa, 20/5 MPa, 35/5 MPa, 50/5 MPa). At each pressure stage a sample was taken. A concentration of the respective emulsion (0.2 %) was prepared for each sample prior to turbidity analysis and particle size measurement.

2.3.3. Impact of particle amount

At constant oil content, a decrease in particle size leads to a significant higher amount of oil droplets. To evaluate whether the amount of particles has an impact on turbidity, particle count calculations were undertaken for two emulsions with a distinct difference in the particle size distribution. Calculations were performed using the standard emulsion (fine emulsion, FE) and the corresponding PE. However, the calculation of the particle amount via the surface weighted mean diameter revealed that PE must be dosed at a concentration, which is 1230 fold higher than the concentration of the FE to include the same amount of particles. Since this is physically impossible an approximation was investigated by diluting both emulsions in ascending concentration (0.1 % w/w, 0.4 % w/w, 0.7 % w/w, 1 % w/w, 2 % w/w and 3 % w/w) to monitor and compare the differences in the increase of turbidity.

2.3.4. Impact of continuous phase

To determine the impact of the continuous phase on the turbidity, the pre- and fine emulsion of the standard emulsion were also prepared in a beverage model solution (PEB and FEB) with 10°Brix and pH 2.6, prepared from 72.7 °Brix inverted sugar syrup and 1.1 % citric acid.

2.3.5. Impact of weighting agent

Whether a weighting agent has an impact on turbidity was evaluated by preparing the standard emulsion with three different oil phases. Weighting agents are widely used in the beverage industry and typically weighted orange oil emulsions give the highest turbidity. The weighted oil phases were prepared by dissolving 30 % w/w of a typical weighting agent (glycerol ester of wood rosin) into MCT oil (MCTW) and orange oil (OOW) respectively on a magnetic stirrer hot plate IKA® C-MAG HS7 (IKA®-Werke GmbH & Co. KG, Staufen, Germany) while heating up to 85 °C and 70 °C respectively. Density and refractive index of the respective oil phase were measured before homogenization. As densities for the different oil phases differed quite remarkably, emulsion formulas were adjusted to a similar volume of the oil phase (7.5 % v/w) rather than a similar weight, which appears to be more correct in terms of turbidity and light scattering (data not shown). In order to achieve emulsions with similar particle size distribution homogenization conditions had to be optimized. As a consequence homogenization pressure had to be adjusted to 35/5 MPa for the emulsion with weighted MCT oil. The emulsions with weighted oil phase were compared to the standard emulsion based on MCT oil. Comparison of emulsions with weighted and unweighted orange was not possible. The density of unweighted orange oil is too low for stable emulsion preparation. For the three emulsions concentrations of 0.1 % w/w, 0.4 % w/w, 0.7 % w/w, 1 % w/w, 2 % w/w and 3 % w/w were made to measure turbidity.

2.3.6. Impact of the type of emulsifier

To determine whether the emulsifier has an impact on the turbidity of an oil-in-water emulsion, emulsions with different emulsifier, but similar particle size distribution and oil load had to be

prepared. Apart from the standard emulsion with GA as classical emulsifier for food grade emulsions, octenyl-succinate derivatized starch (OSA), WPC (80 %) and extract from *Quillaja saponaria* Molina (65 – 90 % saponine, Q) were used. Dosage of each emulsifier was chosen according to the suppliers' recommendation. As WPC-based emulsions were not stable under acidic conditions for a prolonged period of time all emulsions were prepared without preservatives and all analysis were done on the day of preparation. In order to achieve emulsions with similar particle size distribution, it was necessary to adjust formulation and/or homogenization procedure (see Table 2-1).

Table 2-1: Composition and process conditions for the preparation of emulsions with standardized oil droplet size distribution using different emulsifiers (whey protein concentrate 82 % (WPC), *Quillaja* extract (Q), octenyl-succinate derivatized starch (OSA) and gum arabic (GA))

	WPC	Q	OSA	GA
Emulsifying agent [g]	16.70	23.33	88.80	101.25
MCT oil [g]	78.95	88.50	70.90	70.90
Water [g]	389.76	374.25	375.00	375.00
Homogenization pressure (MPa)	25/5	50/5	20/5	50/5
Number of passes	4	4	4	4

Turbidity was compared at 0.2 % and 2 % concentration of standard emulsion representing a typical CSD and a juice containing beverage. Dosage for turbidity measurement had been calculated from the respective formulations to obtain the same oil load (w/w) for all emulsions (data not shown).

2.3.7. Physical characterization of the emulsions

For turbidity measurement emulsions were diluted as outlined above and left to settle for approx. 30 minutes prior to turbidity measurement. Turbidity was measured using a 2100AN IS laboratory turbidimeter DIN EN ISO 27027:2000-04 compatible with infrared light source at 870 nm ± 30 LED (Hach Lange GmbH, Berlin, Germany) with four detectors measuring 90°,

180°, forward and backward scattering. All measurements were done in triplicate and readings are recorded in NTU (ratio) – mode, which is a ratio of the signals of all four detectors.

Refractive index (RI) was measured using a digital refractometer (RX-5000, ATAGO CO., LTD., Tokyo, Japan; accuracy of measurement (nD): 0.00004, LED wavelength: 589 nm, prism: sapphire) at 20 °C. Measurements were done in triplicate. Density was measured using a temperature controlled flexural resonator DMA 4500 Density Meter (Anton Paar GmbH, Graz, Austria) at 20 °C. All measurements were done in triplicate.

Mettler Toledo S220 SevenCompact™ pH meter was used to measure the pH of the beverage test solution (Mettler-Toledo Intl. Inc, Greifensee, Switzerland).

Particle size characterization was done via laser diffraction method using a Malvern Mastersizer 2000 equipped with a dispersion unit Hydro 2000S (both Malvern Instruments Ltd, Worcestershire, UK). For each sample five measurements were made using the following parameters: pump speed was set at 2000 rpm, measurement delay was 2 seconds, measurement time was 10 seconds and 10,000 snaps were taken. For emulsions with different oil phase trials were undertaken to optimize optical parameters. The instrument software provides two quality parameters of the data fit. Optimization means that similar and low values for the residuals and the weighted residuals should be achieved as these parameters indicate the correctness of the distribution calculation. For emulsions with MCT the RI is 1.45 and absorption is 0.001 for both blue and red laser. For MCTW red laser only measurement was chosen due to strong absorbance of the ester gum; the RI is 1.47 and abs. is 0.001. OOW emulsion is as well measured with red laser only; the RI is 1.49. Particle laser diffraction measurement was undertaken and reported following ISO 13320:2009 and ISO 9276-2:2014.

2.3.8. Sensory analysis of the emulsions

In total 22 tests were undertaken to quantify the difference in turbidity perceivable for the panelists at turbidity levels ranging from 100 – 9500 NTU (ratio). Three ranges of turbidity were chosen. Tests at a turbidity of 100 – 250 NTU (ratio) represented the low turbidity range, 900 – 1500 NTU (ratio) the medium turbid range and test performed at turbidity values above 3000 NTU (ratio) represented the high turbidity range. Typical products in the low turbidity

range are non-alcoholic CSDs like lemonade. Juice containing drinks like nectar or whey drinks range usually in the medium turbid range and juices like orange juice yield turbidity around 4000 NTU (ratio). Consequently, for beverage development these three turbidity ranges are of interest. In each turbidity range the samples were chosen at random with turbidity differences defined by best estimate. In case all samples were judged significantly different, turbidity differences were gradually decreased for the following evaluation until samples could not be differentiated any more by the panelists. In case differences were not detected, turbidity differences between samples were gradually increased until the limits for distinction of visual human perception had been identified.

For all sensory tests concentrations of the standard emulsion were used. Dosage for specific turbidity samples were determined and calculated by five point calibration and linear regression. Concentrations from 0.1 % w/w – 6.7 % w/w resulted in turbidity from 110 – 9450 NTU (ratio). Turbidity was controlled for each sample via turbidity measurement as described above. The sample solution was filled into 15 mL screw cap bottles. Sensory analysis was undertaken according to DIN ISO 8587:2010-08 (Sensory analysis – Methodology – Ranking). According to availability of panelists, sets of three to five samples coded with random numbers in random order were presented in complete block design in a dedicated sensory room with blinded windows and daylight imitating illumination. The room was set up according to DIN 10962:1997-10 (Areas for sensory analysis – Specifications of testrooms). The panel consisted of 23 un-trained fellow employees aged 18 to 58 years who self-reported normal or corrected to normal vision. The panel was instructed to rank the samples ascending according to turbidity. Rank 1 being the least turbid, rank 2 more turbid and so forth. The panelists were told to touch the bottle only at the black lid to avoid finger prints on the glass. In case no difference could be detected the panelist had to make a decision on the rank but left a comment. The aim was to rank samples based on the intensity of a single attribute, in this case turbidity. For evaluation rank data were calculated for each sample. In case of tied ranks the mean of both ranks was given to each of the tied ranks. The value of Friedman Test (F) was computed to determine if there were any differences among the samples. For complete block design, F was calculated according to Equation (2-1). In case of tied ranks F was corrected to F' (Equations (2-2) and (2-3)):

$$(2-1) \quad F = \frac{12}{(n \cdot k \cdot (k+1))} \cdot (R_1^2 + R_2^2 + \dots + R_k^2) - 3 \cdot n \cdot (k+1)$$

with n giving the number of panelists, k giving the number of samples and R_{l-k} being the rank sum of the respective sample;

$$(2-2) \quad F' = \frac{F}{1 - \frac{E}{n \cdot k \cdot (k^2 - 1)}} \quad \text{with}$$

$$(2-3) \quad E = (t_1^3 - t_1) + (t_2^3 - t_2) + \dots + (t_i^3 - t_i)$$

with t_{l-i} : number of tied ranks. In the case F_{Test} was higher than F (value for F can be extracted from a table given in ISO 8587:2006), with the chosen level of significance ($\alpha = 0.01$), there were significant differences at least between two samples. The least significant difference (LSD) between two samples, with the chosen risk ($\alpha = 0.05$) was calculated according to Equation (2-4):

$$(2-4) \quad LSD = 1.96 \sqrt{\frac{n \cdot k \cdot (k+1)}{6}}$$

with n giving the number of panelists and k giving the number of samples. In case the difference in rank sums was as large as or larger than the LSD-value, the samples were considered to be significantly different.

2.3.9. Statistical Analysis

All measurements were conducted at least in duplicate. All means, standard deviations, regressions, Friedman Tests and LSDs were calculated in Microsoft® Excel (Microsoft Office 2010).

2.4. Results and Discussion

2.4.1. Impact of particle size

The impact of particle size on the turbidity of an oil-in-water emulsion is presented in Figure 2-1. The corresponding results from particle size analysis are given for all emulsions in Table 2-2. An exponential relationship between the decrease in particle size with the increase in turbidity exists. The exponential regression for the mean volume-surface particle diameter $\bar{x}_{1,2}$ is given by Equation (2-5):

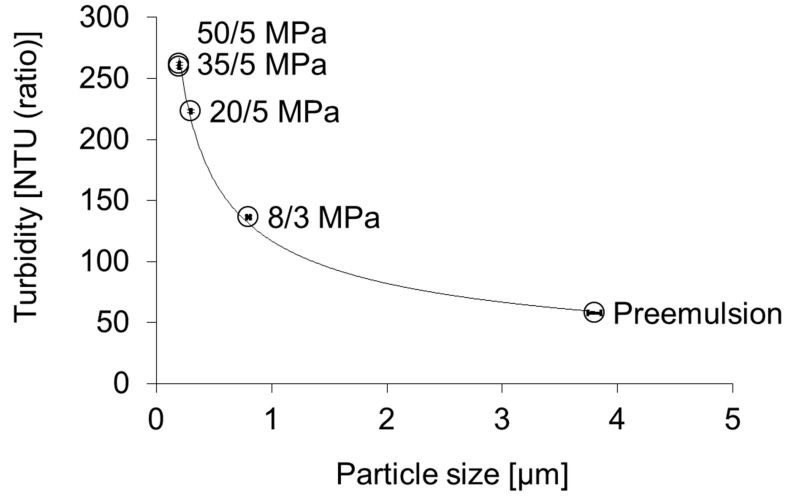


Figure 2-1: Exponential correlation between turbidity [NTU (ratio)] and $\bar{x}_{1,2}$ of 0.2 % w/w emulsions homogenized with increasing pressure ($y = 117.04x^{-0.512}$, $R^2 = 0.998$). All data are means $\pm$ standard deviations for $n = 3$.

$$(2-5) \quad \bar{x}_{1,2}: y = 117.04x^{-0.512}$$

with $R^2 = 0.998$. The exponential regression results from the fact that scattering as the most important parameter determining turbidity, is not only affected by the number of particles, but also their size. Turbidity together with the absorption determines the extinction coefficient (α) of a sample. The intensity, polarization, angular distribution, and fine structure of the scattered radiation are determined by the number of particles, their size, shape, optical constants, and interactions of the molecules in the scattering material (Oster, 1948). Therefore α can be described with Equation (2-6) as:

$$(2-6) \quad \alpha = N\pi R^2 Q$$

where N is the number of droplets per unit volume, R is the droplet radius and Q is the scattering efficiency. Furthermore oil droplet size also affects the scattering efficiency. The scattering efficiency is a function of the wave length of light, the particle radius, the real and the imaginary part of the complex refractive index and the angle of observation θ (Chantrapornchai et al., 1998):

$$(2-7) \quad Q = f(\lambda, R, n, k, \theta)$$

Table 2-2: Particle size distribution results for the different emulsions used for analysis. All data are given as mean $\pm$ standard deviation from analytical replicates for $n = 5$.

Parameter	Pressure [MPa]	$x_{90,3}$ [μm]	$x_{50,3}$ [μm]	$x_{10,3}$ [μm]	$\bar{x}_{1,2}$ [μm]
Particle size	PE	7.8 ± 0.11	4.4 ± 0.07	2.4 ± 0.04	3.8 ± 0.06
	3*8/3	1.8 ± 0.03	0.9 ± 0.02	0.4 ± 0.01	0.8 ± 0.02
	3*20/5	0.7 ± 0.00	0.4 ± 0.00	0.2 ± 0.00	0.3 ± 0.00
	3*35/5	0.5 ± 0.01	0.3 ± 0.00	0.2 ± 0.00	0.2 ± 0.00
	3*50/5	0.4 ± 0.00	0.3 ± 0.00	0.1 ± 0.00	0.2 ± 0.00
Particle count/ cont. phase	PE	6.7 ± 0.2	3.9 ± 0.00	2.2 ± 0.06	3.4 ± 0.06
	FE	0.7 ± 0.00	0.4 ± 0.00	0.2 ± 0.00	0.3 ± 0.00
Weighting agent	MCT	0.7 ± 0.00	0.4 ± 0.00	0.2 ± 0.00	0.3 ± 0.00
	MCT_w	1.2 ± 0.00	0.4 ± 0.00	0.2 ± 0.00	0.4 ± 0.00
	OO_w	0.6 ± 0.02	0.3 ± 0.00	0.1 ± 0.01	0.2 ± 0.00
Emulsifier	GA	0.5 ± 0.00	0.3 ± 0.00	0.2 ± 0.00	0.3 ± 0.00
	OSA	0.9 ± 0.00	0.4 ± 0.00	0.2 ± 0.00	0.4 ± 0.00
	WPC	0.6 ± 0.00	0.3 ± 0.00	0.1 ± 0.00	0.2 ± 0.00
	Q	0.6 ± 0.00	0.3 ± 0.00	0.2 ± 0.00	0.3 ± 0.00

In addition, Figure 2-1 shows that for the sample emulsion the increase in turbidity levels off and maximum turbidity is reached when the volume-surface mean diameter is $0.2 \mu\text{m}$. This is in agreement with previous studies, e.g. for weighted orange oil emulsion maximum turbidity readings were found for mean particle radius of $0.3 \mu\text{m}$ and for uniform latex particles of about $0.6 \mu\text{m}$ (Hernandez & Crandall, 1991; Hernandez & Baker, 1991). The propagation of light is highly dependent on a size parameter $x = 2\pi r/\lambda$. For very small particles with $x \ll 1$ Rayleigh scattering is applied (valid for particles about one tenth of the size of the wavelength; Oster, 1948). In this case just an oscillatory dipole is induced and the medium will appear transparent. Whereas for $x \sim 1$, the scattering of light can be explained with Mie's theory (Farinato & Rowell, 1983). This requires the solution of Maxwell's equations and the description treats the system as a strongly scattering suspension of hard spheres of high refractive index n , dispersed in a

homogeneous medium of refractive index n_0 (Aymard et al., 2000). Mie concluded from his calculations that the particle diameter for which the maximum of scattering is reached is proportional to the wave length and accordingly, the maximum scattering is inversely proportional to the wave length (Mie, 1908). Thus, maximum scattering will occur for a given material, when the particles are about equal in size to the wavelength (Oster, 1948). It needs to be emphasized that all these consideration are based on the assumption of single scattering of monodispersed particles in a non-absorbing medium, which holds not true for the emulsions in the present study. However, for polydisperse systems, as in the case of an oil-in-water emulsion, Dobbins & Jizmagian (1966) found that the mean scattering cross section depends primarily upon the volume to surface ratio and is only weakly dependent on the shape of the size distribution function with validity to mean surface-volume diameters smaller and bigger compared with the wavelength of incident light. It follows for the average scattering efficiency Equation (2-8):

$$(2-8) \quad Q_{av} = \int_0^{\infty} Q(D) * p(D) * D^2 dD / \int_0^{\infty} p(D) * D^2 dD$$

Turbidity will then be given by Equation (2-9):

$$(2-9) \quad \tau = \frac{3}{2} \frac{c}{\rho_m} \frac{Q_{av}}{D_{32}}$$

Therefore the mean volume-surface diameter is suitable for the characterization of polydisperse systems like the emulsions examined in the present study.

2.4.2. Impact of particle amount

The impact of particle amount was evaluated with an approximation via the increase in turbidity between concentration steps of the pre- and fine emulsion of the standard emulsion. With increasing dosage, particle size distribution does not change, but the number of particles does. As depicted in Table 2-2, the PE had a $\bar{x}_{1,2}$ of 3.4 μm and FE had a $\bar{x}_{1,2}$ of 0.3 μm . Furthermore the PE had a $x_{10,3}$ of 2.2 μm and the FE had a $x_{90,3}$ of 0.7 μm , which means that the particle size distributions of the two emulsions had no overlap. Figure 2-2 shows the correlation between the concentration of the cloud and turbidity for PE and FE respectively. The intercept was set to zero, because there will not be turbidity without added emulsion. As the instrument for turbidity measurement is calibrated linear according to formazine standard concentration, it is assumed to

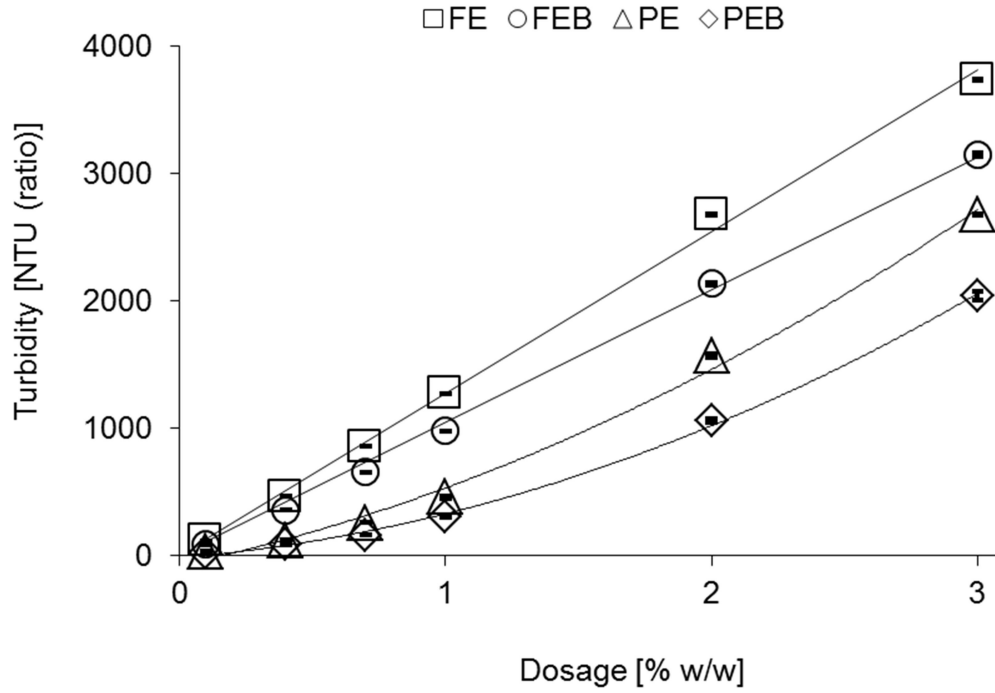


Figure 2-2: Comparison of turbidity [NTU (ratio)] between a fine ($\bar{x}_{1,2} = 0.2 \pm 0.00 \mu\text{m}$) and a coarse ($\bar{x}_{1,2} = 3.4 \mu\text{m} \pm 0.06 \mu\text{m}$) emulsion in deionized water (FE, CE) and beverage model solution (FEB, CEB) with 10 °Brix and pH = 2.6 including linear regression for FE and FEB and polynomial regression for CE and CEB. All data are means $\pm$ standard deviations for $n = 3$.

likewise detect a linear correlation for these oil-in-water emulsions. This is the case for FE with a coefficient of determination of $R^2 = 0.997$. For PE on the other hand a polynomial correlation of second order is found with $R^2 = 0.995$. The corresponding regressions are given in Table 2-3. For 0.1 % dosage turbidity is 30 ± 0.21 NTU (ratio) for the pre-emulsion compared to 113 ± 0.58 NTU (ratio) for the fine emulsion. These results demonstrate the impact of particle size, as the particle size distribution of FE induces maximum scattering, whereas the scattering efficiency of the much larger particles of PE is lower for reasons discussed above. The increase in turbidity on the other hand is steeper for PE due to the polynomial correlation, although much more particles are added to FE. The impact of particle count on turbidity can possibly not be separated from the particle concentration in beverage emulsions. But it can be observed that the scattering of larger particles is more dependent on particle concentration than that of small particles. This might be due to the fact that the size parameter becomes $\gg 1$ for PE and Mie theory is extended to geometrical optical laws (Oster, 1948; Blaunstein et al., 2010).

Table 2-3: Regression for the pre-emulsion (PE) and corresponding standard emulsion (FE) in deionized water compared to preparation in beverage model solution (PEB, FEB, 10°Brix, pH 2.6). * RI data are means $\pm$ standard deviations for $n = 3$.

Sample	RI(cont. phase)*	regression	R ²
PE	1.333 $\pm$ 0.00	$y = 162.1x^2 + 444.43x - 76.319$	0.9954
PEB	1.348 $\pm$ 0.00	$y = 173.31x^2 + 170.99x - 16.658$	0.9989
FE	1.333 $\pm$ 0.00	$y = 1269.9x$	0.9972
FEB	1.348 $\pm$ 0.00	$y = 1042.7x$	0.9978

2.4.3. Impact of continuous phase

Figure 2-2 shows the impact of the composition of the continuous phase on turbidity in the diluted state. When diluted in a beverage model solution the increase in turbidity follows, as well as for FE and PE, a linear correlation for FEB ($R^2 = 0.998$) and a polynomial correlation for PEB ($R^2 = 0.999$). But total turbidity is lower than in systems with water as continuous phase. This effect can be explained with the increase in RI of the continuous phase (see Table 2-3).

The RI describes the phenomenon that light travels at different speeds in different media. As a consequence a beam of light is bent or refracted upon changing the speed when entering a medium with different optical properties (Kuchling, 2007). As a consequence in case the RI of the medium is equal to that of the solute, the RI ratio is zero and the emulsion appears transparent (Oster, 1948). The latter has been shown by Chantrapornchai et al. (2001) who rendered RI ratio of an oil-in-water emulsion by increasing the RI of the continuous phase with glycerol. In the present study citric acid and inverted sugar syrup increased the RI of the continuous phase from 1.333 to 1.348 which renders the RI ratio by 0.015 and led to a decrease in turbidity by 20 %. Similar findings were reported by Benitez et al. (2007), who observed that the turbidity decreased with the presence of glucose in the continuous phase of apple juice.

2.4.4. Impact of weighting agent

Investigating the impact of the oil phase, i.e. the refractive index of the dispersed phase, on turbidity, all three emulsions show a linear correlation between concentration and turbidity with R^2 above 0.99 (Figure 2-3). From the slope of the correlation lines it can be derived that turbidity of an emulsion containing OOW (weighted orange oil) increases 1.7 fold compared to MCT (medium chain triglyceride) and still 1.3 fold compared to MCTW (weighted MCT). As a consequence at low concentration in a range similar to a typical cloud emulsion tremendous differences in turbidity occur. At 0.1 % concentration turbidity amounted to 115 ± 0.58 NTU (ratio) in MCT-based emulsions, 139 ± 0.00 for MCTW-based emulsions and 230 ± 0.58 for OOW-based emulsions. Table 2-4 shows that the refractive index changes with the addition of 30 % weighting agent into the oil phase. The increase of the refractive index is from 1.45 to 1.47 for MCT and from 1.47 to 1.49 for orange oil. With increasing refractive index of the oil phase

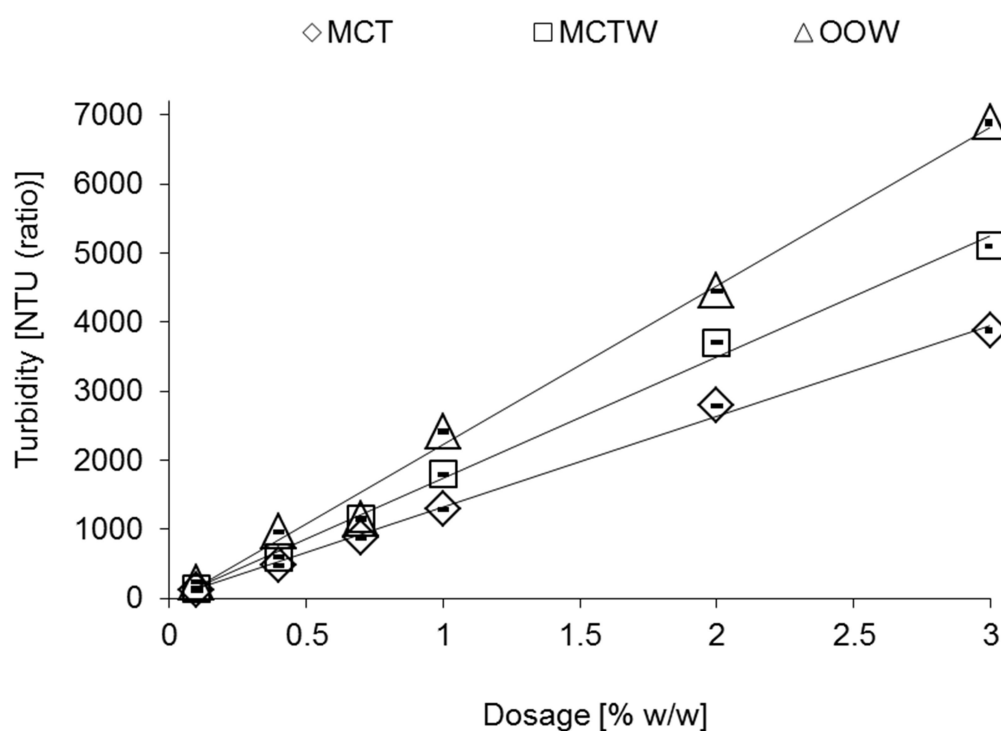


Figure 2-3: Correlation of turbidity [NTU (ratio)] and concentration [% w/w] of oil-in-water emulsion with different oil phase (MCT = medium chain triglyceride; MCTW: MCT weighted with 30 % w/w glycerol ester of wood rosin; OOW: respectively weighted orange oil). All data are means $\pm$ standard deviations for $n = 3$.

the difference between $RI_{\text{dispersed phase}}/RI_{\text{continuous phase}}$ of the emulsions increases by 0.02 for each oil phase. As already described for the continuous phase the highest scattering efficiency was found at high RI ratios (Chantrapornchai et al., 2001). Kaufman & Garti (1984) also found a significant increase in opacity for spectroscopic 1:1000 concentrations when adding EG to similar oil-in-water emulsion. Due to the addition of weighting agent the change of the RI ratio is more pronounced than for the alteration of continuous phase in the trials above. Respectively, more pronounced results were expected and could be confirmed. From the slopes for the linear regressions given in Table 2-4 can be derived that with an increase in RI ratio by 0.02 at the same concentration, the turbidity is increased by 32 %. With an increase of RI ratio of 0.04 the turbidity is increased by 72 %.

Table 2-4: Surface weighted mean diameter $\bar{x}_{1,2}$ and refractive index (RI) of the oil phase for oil-in-water emulsions with medium chain triglyceride (MCT), MCT weighted with 30 % w/w glycerol ester of wood rosin (MCTW) and respectively weighted orange oil (OOW). * Particle size is given in means $\pm$ standard deviations for $n = 5$, **RI is given in means $\pm$ standard deviations for $n = 3$.

sample	$\bar{x}_{1,2}$ [μm]*	$RI_{\text{oil phase}}$ **	Linear	
			regression	R^2
MCT	0.3 ± 0.00	1.45 ± 0.00	$y = 1317.1x$	0.997
MCTW	0.4 ± 0.00	1.47 ± 0.00	$y = 1744.3x$	0.996
OOW	0.2 ± 0.00	1.49 ± 0.00	$y = 2263.5x$	0.993

2.4.5. Impact of emulsifier

The impact of the type of emulsifier on the turbidity is depicted in Figure 2-4. At a concentration of 0.2 % only small differences in turbidity occur. The values ranged from 340 ± 0.6 to 380 ± 0.6 NTU (ratio). In contrast, at a concentration of 2 % differences in turbidity are more pronounced and ranged from 3320 ± 21.0 NTU (ratio) for Q to 4150 ± 6.8 NTU (ratio) for OSA. At both concentrations the emulsion stabilized with Quillaja extract showed the lowest turbidity

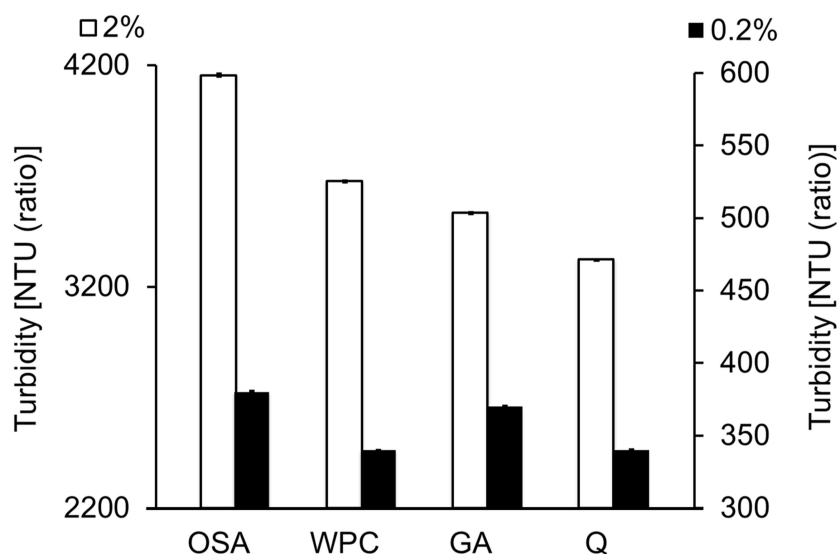


Figure 2-4: Turbidity of 0.2 % and 2 % concentrations of emulsions with different emulsifiers (OSA = octenyl succinate derivatized starch, WPC = whey protein concentrate, GA = gum Arabic, Q = Quillaja saponaria Molina extract). All data are means $\pm$ standard deviations for $n = 3$.

and emulsions stabilized with OSA the highest turbidity. The emulsions were prepared with the same oil phase and under conditions resulting in a similar oil droplet distribution. As can be seen in Table 2-2, the $\bar{x}_{1,2}$ ranges between 0.2 – 0.4 μm . Therefore all emulsions should be in the size range which induces maximum scattering effects so that the oil phase and the particle size should not be a major influencing factor. Ray et al. (1983) stated that the refractive index of the dispersed phase may be different from that of the bulk oil. But he made as well the assumption that the thickness of the interface is small enough, relative to the wave length of the incident light, that the emulsified oil droplet behaves optically as a single dispersed phase (Ray et al., 1983). In the present study a possible impact of the emulsifier was only observed at high emulsion concentrations. The differences in turbidity at higher dosage can be explained by excess emulsifier, as scattering actually happens in every suspension because each molecule acts as scattering center (Van de Hulst, 1981). Also excess emulsifier that forms micelles or aggregates in the continuous phase, shows scattering and its impact is therefore more pronounced at higher concentrations. This can be derived from the chemical structure of the emulsifiers used in the present study. The saponins in the Quillaja extract have a molecular weight of 1800 – 2000 Da and form micelles of approximately 15 nm (Rigano et al., 2009) This should be too small to scatter light effectively (as explained above) and explains the low turbidity for the Quillaja

emulsion. Whereas OSA ($1.2 - 2.6 \cdot 10^5$ Da), WPC ($1.6 \cdot 10^4 - 2.5 \cdot 10^5$ Da) and GA ($3.1 \cdot 10^5$ Da) have much higher molecular weights (Dokić et al., 2008; Wang & Lucey, 2003; Dickinson et al., 1991). The differences in turbidity between the latter three might then result from different aggregation.

2.4.6. Sensory evaluation

The result of the sensory evaluation clearly shows that detectable differences in turbidity depend on the turbidity range studied (Figure 2-5). For each range of turbidity the minimum turbidity difference in NTU (ratio) is given, which was required to significantly distinguish two samples from each other. As can be seen, in the low turbidity range panelists were able to differentiate between samples with a difference of 25 turbidity units, i.e. a sample with 125 NTU (ratio) has been perceived as significantly more turbid than a sample with 100 NTU (ratio). While the turbidimeter is calibrated linear according to concentration, the visual human perception of the

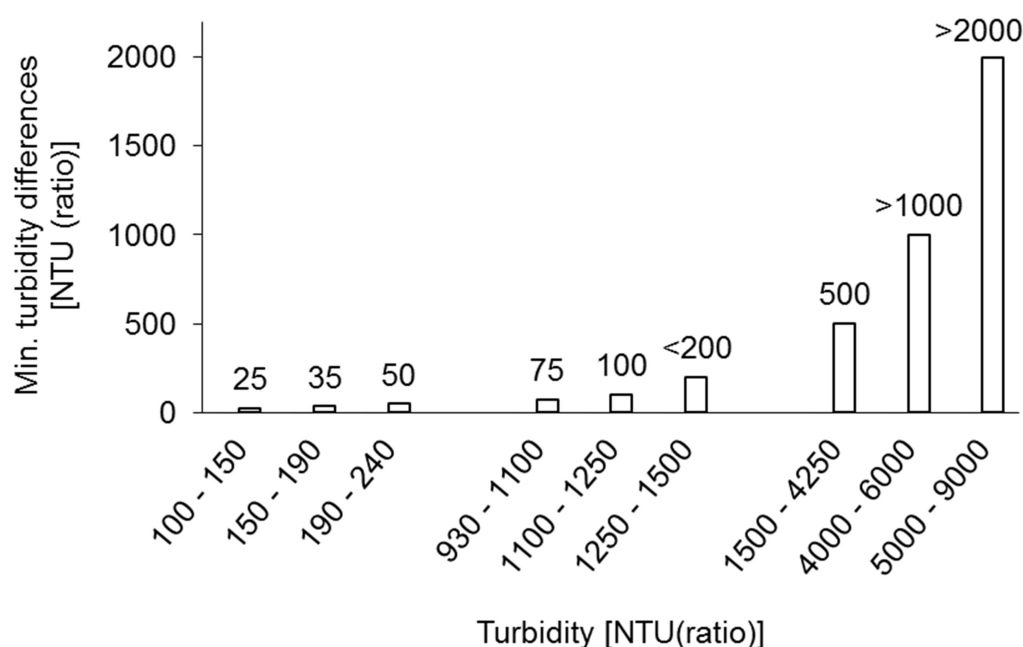


Figure 2-5: Minimum turbidity differences between two samples for a given turbidity range detected by human visual perception; analyzed by Friedman Test ($\alpha = 0.01$) and least significance difference ($\alpha = 0.05$).

sample emulsion is different for each range of turbidity. While at low turbidity an average increase of 21 – 24 % from the initial turbidity is needed to recognize a difference, at a medium turbidity level an increase of 7 % was already recognized. At a turbidity level of around 4000 NTU (ratio) it becomes more and more difficult to distinguish between samples. An increase in turbidity of more than 40 % was required to induce significant differences. Nevertheless, great values of turbidity could still be significantly distinguished and sorted according to ascending turbidity. According to Stevens' power law the subjective magnitude to an intensity of a physical stimulus is related by Equation (2-10):

$$(2-10) \quad E = k (R - R_0)^n$$

with E being the subjective magnitude, R is the stimulus intensity and R_0 is the threshold for the given stimulus (Stevens, 1957). With this law Stevens described the results of experiments on loudness, brightness, vibration on the skin, and warmth and cold applied to the arm. In the present study no magnitude estimation was undertaken, but a ranking test to find the least differences detectable. A correlation of the stimulus intensity (turbidity) to the minimum turbidity differences is depicted in Figure 2-6. As opposed to magnitude estimation, for the human visual $R^2 = 0.997$. For the development of products with a specific turbidity these data provide valuable

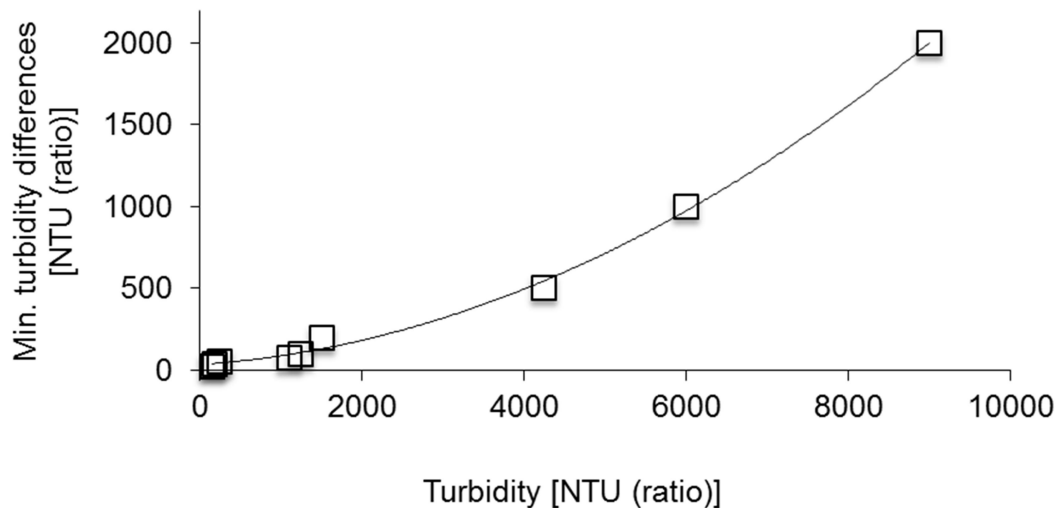


Figure 2-6: Relationship between minimum turbidity differences detected by human visual perception and upper turbidity value for which this difference is detectable ($y = 2E-05x^2 + 0.0328x + 34.89$, $R^2 = 0.9976$).

information as turbidity measurement allows the estimations of the human response. It may be perception of turbidity in beverage systems and the turbidity range investigated, a polynomial relationship can be found withdeduced, if product deviations will be detected by consumers. On the other hand these results give indications which changes in the visual appearance need to be achieved in product formulation for a specific more turbid or a specific less turbid product.

2.5 Conclusion

The sensory evaluation of the human visual perception of turbidity showed that in contrast to the instrumental reading the human response to turbidity is not linear. Visual perception is most sensitive to turbidity differences in the range between 1000 – 1500 NTU (ratio). At very high turbidity values a difference of more than 2000 TU in NTU (ratio) was needed to detect significant differences between samples. With this knowledge on visual perception of turbidity and the factors determining turbidity, turbidity can be tailored in product development according to the customer requirements and in quality control to define acceptable variations in optical appearance.

Concerning the parameters affecting turbidity in beverage emulsions, particle size was the most important factor affecting turbidity with an exponential correlation until a maximum turbidity is reached. It was shown that a maximum turbidity occurs at a mean volume-surface diameter of 0.2 μm for the oil droplet size. This is also desirable to the physical stability and should be controlled in this respect. In diluted form, in a concentration of the cloud typical for a CSD, a change in the refractive index of the oil phase may allow the alteration of turbidity by up to 30 % which makes the refractive index the most promising parameter for turbidity adjustment. The refractive index of the continuous phase of the final beverage affects turbidity in a similar manner. But, this cannot be considered a suitable strategy to modify the turbidity of a beverage, since the properties of the continuous phase are defined through the final application. An effect of the type of emulsifier on turbidity was only detected at high emulsion concentration and is attributed to excess emulsifier in a dispersed state. This factor is not suitable to control turbidity in beverages, since the effect was below the minimum difference panelist could distinguish at a typical level of turbidity. In summary both, formulation and process conditions must be considered when tailoring the optical properties of an oil-in-water emulsion towards the desired appearance of

beverages. Taking into consideration recent trends in the beverage industry like clean labeling, future research should evaluate the functionality of alternative cloud systems like fiber-based microparticle suspensions or surfactant free- oil-in-water emulsions.

2.6 Acknowledgment

The authors would like to thank Gerhard Krammer and the Symrise AG for supporting this work.

3. Manuscript II: Pickering emulsions in foods – opportunities and limitations

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This is an Accepted Manuscript of an article published by Taylor & Francis in *Critical Reviews in Food Science and Nutrition* on 30 October 2017, available online:

<https://www.tandfonline.com/doi/full/10.1080/10408398.2017.1290578>

3.1. Abstract

In order to critically discuss the potential of Pickering-type emulsions in food applications this review provides the theoretical background of the stabilizing mechanisms, the resulting requirements for particles to stabilize these systems and the limitations resulting from these fundamental considerations. Food grade particle systems investigated in the past are presented. It becomes obvious that with a proper choice of a particle type, oil-in-water as well as water-in-oil emulsions can be achieved. For highly viscous products, products with a high internal phase volume and foams Pickering particles offer alternatives to commonly used surfactants. Pickering emulsions might be able to offer new approaches for fat reduction as well as encapsulation and sustained release of active ingredients. Nevertheless, a major part of successful systems have been achieved with silica or modified silica particles, which is not in line with the consumer demand for clean label, natural systems or not even food grade. However, the intriguing possibilities motivate and justify future research on the identification of new suitable ingredients, improvement of existing formulations and identification of new fields of application.

Keywords: Pickering emulsions, food, particles, stability

3.2. Introduction

Emulsions generally consist of small spherical droplets of one liquid dispersed in another immiscible liquid stabilized through a surface active compound. These systems are thermodynamically unstable and tend to breakdown over time through aggregation and coalescence of the dispersed phase (McClements, 2012a). Other limitations inherent to conventional emulsions include limited stability to pH, salt, heating, dehydration, freezing and chilling (Guzey & McClements, 2006).

Pickering emulsions date back to 1903 when Ramsden first discovered the stabilizing effect of particles in emulsions and bubbles (Ramsden, 1903). Named after Pickering, who found similar results a few years later (Pickering, 1907) it took a while before scientists regained interest in particles at interfaces (Chevalier & Bolzinger, 2013). They came into focus when researchers tried to overcome the limitations of conventional emulsions and extensive research was performed during the past ten years (Binks & Horozov, 2006; Leal-Calderon & Schmitt, 2008).

Main advantage of particle-stabilized systems is their superior stability against coalescence (Frelichowska et al., 2009). Another intriguing idea behind these systems is their surfactant free character (Leal-Calderon & Schmitt, 2008). Surfactants known to be environmentally problematic (Braisch et al., 2009) or showing adverse effects (Chevalier & Bolzinger, 2013) can be replaced, which is highly relevant for medical and skincare applications (Sadeghpour et al., 2013). With respect to food applications, surfactant-free formulations meet the demand associated with the clean label trend to create environmentally friendly solutions and a commercial need to make food labels ‘green’ (Green et al., 2013; Kalashnikova et al., 2011). Another intriguing fact is that particle-stabilized systems may be produced by standard emulsifying devices which would allow to easily adapt new products in existing production lines.

Two review articles have recently been published on Pickering emulsions in foods, which either focus on the physical chemistry of Pickering emulsions or on food-grade particles with a potential to serve as Pickering particle (Dickinson, 2012b; Tavernier et al., 2016). We feel that there is a need to combine these approaches and thus focus on a critical discussion of the possibilities and limitations of the usage of Pickering particles for the food industry. To get a better understanding the physics behind Pickering and the mechanisms of stabilization are discussed in detail. Based on these considerations the influence of other surfactants in particles stabilized systems is outlined, since food systems are usually complex systems and particles and surfactants may coexist (Gosh & Rousseau, 2011; Dickinson, 2013). Important recent developments in food grade Pickering particles, not covered by the literature cited, have been included like the recent discussion of hydrophobins. Food-grade particles are screened for their potential for Pickering stabilization and discussed with respect to possible applications for the food industry.

3.2.1. Stabilizing mechanisms in Pickering emulsions

Particles are able to stabilize emulsions by providing a physical barrier to droplet coalescence (Tambe & Sharma, 1994b). If the particles are neither soluble in water nor in oil they will accumulate at the interface. Depending on the wettability of the particles they either immerse more into one phase than into the other (Binks, 2002; Aveyard et al., 2003; Dickinson, 2012b).

The Young equation, described in Equation (3-1), for the determination of the contact angle θ between the three phases describes the situation:

$$(3-1) \quad \cos \theta = (\gamma_{p/o} - \gamma_{p/w}) / \gamma_{o/w}$$

where $\gamma_{p/o}$ is the particle-oil interfacial tension, $\gamma_{p/w}$ is the particle-water interfacial tension and $\gamma_{o/w}$ is the oil-water interfacial tension (Owens & Wendt, 1969; Binks & Clint, 2002)

At a contact angle of 90° a particle is immersed in equal shares into each phase as demonstrated in Figure 3-1.

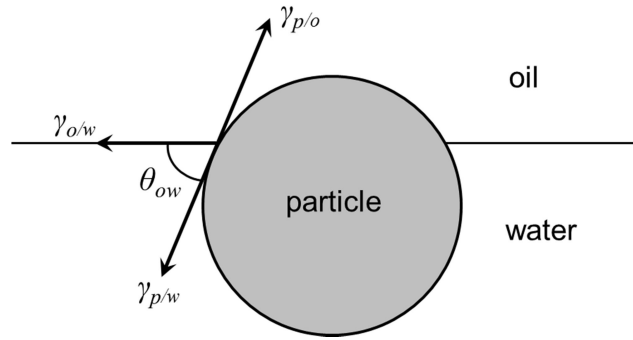


Figure 3-1: Schematic diagram of a spherical solid particle at an oil-water interface showing the various interfacial energies and the contact angle measured into the water phase with the particle-oil interfacial tension $\gamma_{p/o}$, the particle-water interfacial tension $\gamma_{p/w}$ and the oil-water interfacial tension $\gamma_{o/w}$ the oil-water contact angle θ_{ow} (adapted with permission from Binks, B. P.; Clint, J. H. (2002): Solid Wettability from Surface Energy Components. Relevance to Pickering Emulsions. In *Langmuir* 18 (4), pp. 1270–1273. Copyright 2002 American Chemical Society).

If the particle is more hydrophilic the particle is predominantly wetted by the aqueous phase and the contact angle is below 90° (Binks, 2002; Aveyard et al., 2003; Hunter et al., 2008; Leal-Calderon & Schmitt, 2008). Because of the large contact area of particles with water the interface will get a curvature towards the oil phase, which means that rather an oil-in-water emulsion will be formed (Binks, 2002; Binks & Clint, 2002). If the particle is more lipophilic the immersion preferentially occurs into the oil phase and the interface bends towards the aqueous phase, which will result in a water-in-oil emulsion.

Once attached to the interface, the energy needed to remove a particle is given by the free energy of desorption (ΔG_D):

$$(3-2) \quad \Delta G_D = \pi r^2 * \gamma_{o/w} * (1 - \cos \theta)^2$$

with r being the radius of the Pickering particle

From Equation (3-2) it can be derived that the most important factor is particle size as $\Delta G_D \sim r^2$. With increasing particle size, the binding energy increases. Nevertheless even for nano-sized particles ($r \sim 5 - 10$ nm) the free energy of desorption ΔG_D is well above 10 kT, with k being the Boltzman's constant and T the absolute temperature, and particle absorption can be considered irreversible (Binks 2002; Leal-Calderon & Schmitt, 2008; Dickinson, 2010; Sadeghpour et al., 2013). Because of the high binding energy of the particles at the interface, emulsions with large droplet size show high stability against coalescence. Pickering emulsions with an oil drop diameter of 100 μ m or even larger are reported to be stable against coalescence for several months (Timgren et al., 2013; Li et al., 2013; Binks & Lumsdon, 2000).

The above explained mechanism mainly governs Pickering stabilization, but other effects also contribute to the stability. Lopetinsky et al. (2006) summarizes possible configurations of particles at solids-stabilized interfaces. Figure 3-2(a) shows the complete coverage of the droplet surface with particles as one possibility of particle adsorption. In this case a bilayer of particles prevents coalescence of the droplets upon collision. The stabilizing effects are depicted in Figure 3-2(i)–(iv) with (i) and (ii) both being sterical and (iii) and (iv) describing effects based on thin film properties.

Figure 3-2(i) depicts suppression of coalescence as a result of the balance between the gain in free energy upon desorption of particles and the reduction of free energy through shrinkage of the surface area upon coalescence. In case two droplets show coalescence the interfacial area decreases. This implies that particles need to be removed from the interface. The free energy of desorption is far greater than the thermal energy released upon reduction of the interface.

This is an important reason why particles represent such a strong barrier against coalescence. The mechanism was very well demonstrated in experiments of pending drops. Visual observation of inflated and deflated pending drop of silicone oil covered with hydrophobized silica revealed the virtually irreversible particle attachment. No particles were released but the interface crumpled together upon contraction of the drop volume (Asekomhe et al., 2005). Xu et al. (2005) obtained similar results for a sessile water drop covered with polystyrene particles against decane.

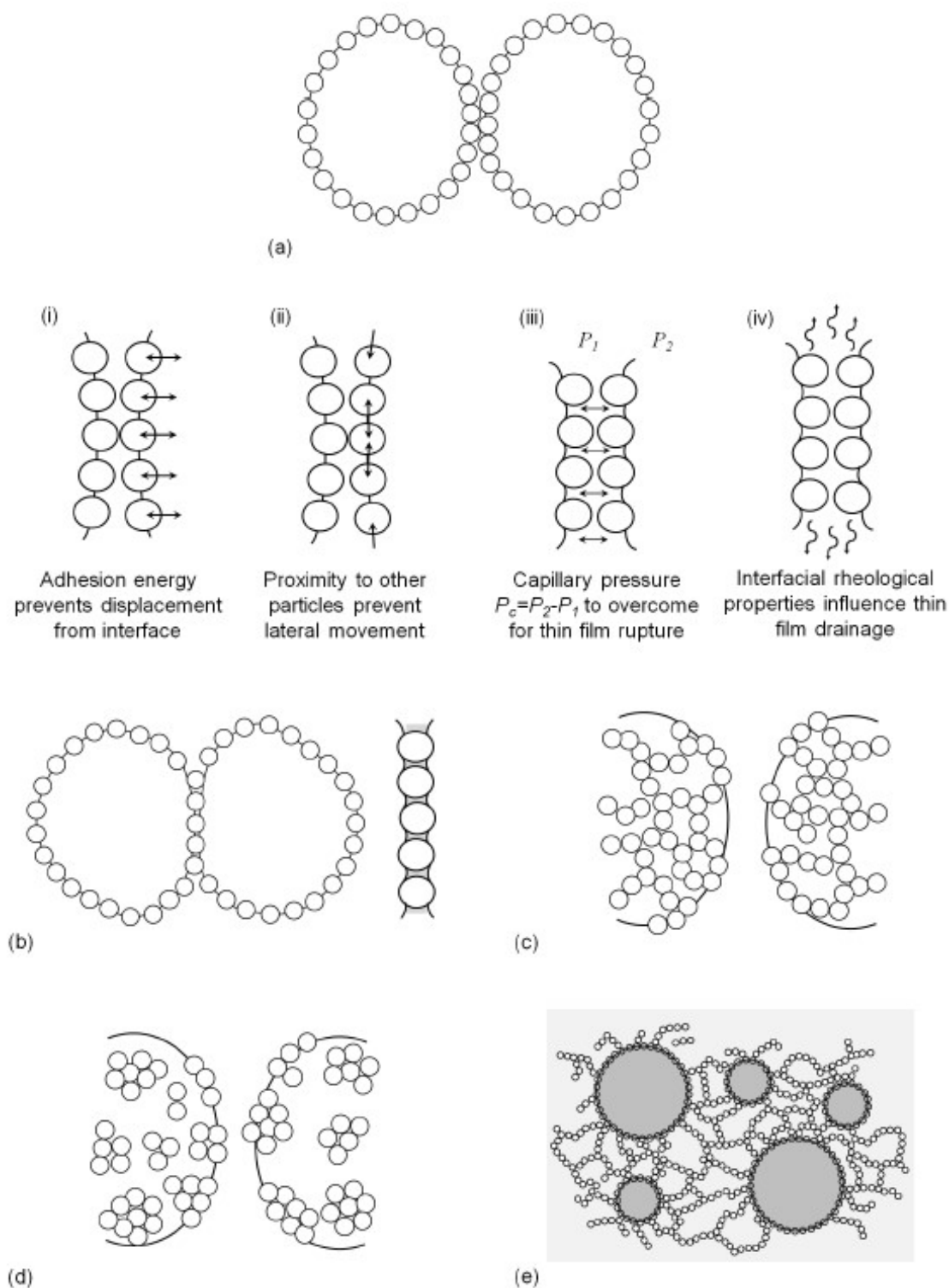


Figure 3-2: Possible configurations of particles in solids-stabilized emulsions, (a)–(e), and the underlying mechanisms responsible for stability (i)–(iv), (adapted with permission from Lopetinsky, Robert J.G.; Masliyah, Jacob H.; Xu, Zhenghe (2006): *Solids-Stabilized Emulsions: A Review*. In Bernard P. Binks, Tommy (ed.) Horozov (Eds.): *Colloidal Particles at Liquid Interfaces*: Cambridge University Press, p. 194. Copyright 2006 Cambridge University Press).

Figure 3-2(ii) depicts the particle strength of aggregation preventing lateral displacement of particles at the interface. The force to laterally displace a particle along the interface is much smaller than to remove it from the interface and is strongly dependent from particle concentration (Tambe & Sharma, 1995). It was found that loosely packed particles provide little resistance to lateral displacement, which may result in emulsions susceptible to shear induced coalescence (Stancik et al., 2003; Whitby et al., 2011). Another stabilizing mechanism is the capillary pressure of the thin film of continuous phase between particles shown in Figure 3-2(iii). Since the force to displace the particles from the interface is too high, particles will approach and contact each other when droplets collide due to Brownian motion. A thin film of continuous phase will be held between the particles pores. In case of complete coverage of the interface with particles, the thin film is stable as long as the pressure inside the film does not exceed the maximum capillary pressure. Another less important aspect with capillary pressure is contact angle hysteresis (Yan & Masliyah, 1996). This is the difference between the contact angle formed by a liquid advancing, θ_a , and receding, θ_r , over a solid surface (i.e. $\theta_h = \theta_a - \theta_r$). Since the capillary pressure is dependent on the contact angle the pressure needed for thin film rupture changes with different contact angles. Depending on the type of liquids and particles these differences determine which type of emulsion will be formed. This hysteresis is caused by several phenomena including surface roughness, surface chemical heterogeneity and adsorption of impurities from the liquid phases (Lopetinsky et al., 2006). Contact angle hysteresis might be the driving factor for those cases where the mixing protocol, and accordingly initial particle location was the driving factor for determining emulsion type.

Figure 3-2(iv) represents the rheological properties of the interface influencing thin film drainage. Tambe & Sharma (1994a) reported that the rheological properties of the interface change with increasing particle concentration. With increasing particle concentration film drainage is more and more influenced by inter-particle interactions (elastic effects). This leads to an interface with viscoelastic properties, which positively affects steric hindrance and thus improves stability against film drainage and coalescence. Again this is only valid for dense particle layers.

Stable interfaces with less dense particle coverage were observed and are depicted in Figure 3-2(b)–(d). A typical phenomenon reported is bridging as demonstrated in Figure 3-2(b) where a single particle layer stabilizes the interface between two droplets. Ashby et al. (2004), Stancik & Fuller (2004), Stancik et al. (2004), Fuller et al. (2006) and Xu et al. (2007) presented images of a

water drop covered completely by polystyrene particles pendant in decane approaching a planar decane-water interface. The two liquid surfaces were virtually irreversibly bridged together by a particle monolayer. The pendant drop could not be detached without breaking it up and when doing so forming a small particle stabilized water droplet formed which resided at the interface. Horozov et al. (2005) suggested that bridging is a bilayer to monolayer transition, which occurs when the capillary pressure exceeds the strength to withstand lateral movement at the interface. But French et al. (2015) came to the conclusion that bridging mainly depends on the factor whether there are enough particles to cover the created surface. This is also supported by Bon & Colver (2007). In their study, the degree of bridging increased with increasing shear rate (smaller oil droplets, more surface to cover) and decreased with increasing particle volume fraction. Lee et al. (2012) were able to form a gel-like emulsion solely stabilized by bridged monolayers of silica microspheres demonstrating that bridging does not necessarily impair stability.

Figure 3-2(c) shows the formation of a 2D network of particles on the droplet surface. Steric hindrance is sufficient to stabilize an emulsion, if the strength of aggregation of the particles prevents displacement at the interface and the energy of adsorption prevents removal from the interface. Electrostatic interactions and steric hindrance are mainly responsible but are not fully understood yet (Lopetinsky et al., 2006). Vignati et al. (2003) suggest monolayer bridges and particle redistribution preventing coalescence and film drainage from observations made from high resolution pictures of advancing sparsely covered particles. Alternatively, particle cluster formation at the interface may occur as depicted in Figure 3-2(d). Aveyard et al. (2000) observed clustering of polystyrene spheres upon addition of anionic, cationic and non-ionic surfactants. The authors conclude that neutralization of charge cannot cause the effect and give the change in interfacial tension as reason for cluster formation.

Last but not least 3D network formation (Figure 3-2(e)) extending through the continuous phase was observed improving stability by preventing droplet to droplet contact (Torres et al., 2007; Chen et al., 2011; Juárez & Whitby, 2012; Lee et al., 2012; Nesterenko et al., 2014; Fuma & Kawaguchi, 2015). If particle charge allows aggregation, this formation is mainly driven by particle concentration. In such cases usually a dense layer of particles at the interface exists stabilizing through above explained mechanisms Figure 3-2(i)–(iv). Excess particles provide additional stabilization by preventing contact between droplets and increasing viscosity of the continuous phase.

3.2.2. Factors influencing formation and stability of Pickering emulsions

From the mechanisms outlined above factors influencing the formation and stability may be derived. The majority of studies have been performed using silica particles and results of these studies can be used to derive a summary of factors affecting the performance of a Pickering-stabilized system. In summary, formation of particle stabilized emulsions depends on the particle wettability, -concentration, -size, on the packing density and shape of particles, on the pH of the aqueous phase and presence of additives (Tambe & Sharma, 1993).

Suitable particle wettability as a requirement for successful Pickering stabilization has already been described above. Different approaches have been described to modify the wettability to generate particles with defined properties. For example mild drying changed the wettability of silica particles because of decreased particle hydration (White et al., 2011). However, the majority of work has been done through combination of particles with other surfactants, which will be discussed in more detail in another chapter.

In order to form a layer around a droplet, another pre-requisite is the particle size. To form a stable emulsion the particles need to be at least one magnitude smaller than the droplets. Thus, the particle size of the Pickering particles determines the minimum achievable oil droplet diameter. Köhler et al. (2010) prepared an emulsion with 3 % hydrophilic silica particles and 20 % corn oil. Focus of the study was to investigate the impact of primary particle size and homogenization conditions. Non-creaming emulsions from 12 nm sized particles with resulting droplet sizes of were produced compared to Use of 12 nm sized particles resulted in non-creaming emulsions with a droplet size of $x_{90,3} \sim 10 \mu\text{m}$ compared to $\sim 100 \mu\text{m}$ when using 200 nm sized particles.

Particle concentration affects particle packing at the interface, which is crucial for emulsion stability. For oil phases with partly water soluble substances, Juarez & Whitby (2012) defined a minimum particle concentration needed to prevent Ostwald ripening. Binks & Whitby (2004) demonstrated that particle concentration also affects the average drop diameter in the emulsion. With increasing particle concentration emulsion droplet size decreased. Frelichowska et al. (2010) found a similar correlation and concluded that for predicting success in emulsification the

particle to oil ratio is more useful than only particle concentration. The interfacial stabilization also depends on the density of packing of the adsorbed layers of nano- or microparticles (Capron & Cathala, 2013). Several models were used to predict particle concentration at the interface. One approach uses a simple mass-balance model, based on the argument, that particles show very similar behavior to surfactants when electrostatic repulsion is suppressed (Tcholakova et al., 2008). Assuming coalescence of the drops until the particle adsorption reaches a certain threshold value Γ^* and that nearly all particles adsorb on the droplet surface, the mean volume-surface diameter correlates with the initial particle concentration following Equation (3-3):

$$(3-3) \quad d_{32} \approx \frac{6\Phi}{(1-\Phi)} \frac{\Gamma^*}{C_{INI}} = \frac{6\Phi}{(1-\Phi)} \frac{\theta^* \Gamma_M}{C_{INI}}$$

$C_{SER} = C_{INI}$ (emulsifier in the continuous phase)

where C_{INI} is the initial particle concentration in the continuous phase, C_{SER} is the concentration of particles left in the aqueous phase after emulsification, Φ is the volume fraction of the dispersed phase, Γ^* is the threshold adsorption, Γ_M is a close packed monolayer of particles and $\theta^* = \Gamma^* / \Gamma_M$ is the dimensionless threshold surface coverage (Tcholakova et al., 2008)

In this model the mean droplet diameter is proportional to the inverse particle concentration and strongly dependent on the volume fraction of the dispersed phase. The predictions of this model could be confirmed for emulsions with low particle concentration and suppressed electrostatic repulsion.

Another approach is to calculate the needed volume of particles to stabilize a certain surface. Assuming a fixed volume of oil and average droplet size, one may calculate the total drop surface area (S_d) based on Kalashnikova et al. (2011):

$$(3-4) \quad S_d = n_o \times \pi d_o^2 = \frac{6V_{oil}}{\pi d_o^3} \times \pi d_o^2 = \frac{6V_{oil}}{d_o}$$

where n_o is the number of oil drops, d_o is the sauter mean drop diameter and V_{oil} the volume of oil emulsified

Assuming a contact angle of 90° , the theoretical maximum surface that can be covered by the particles S_p , is defined by:

$$S_p = n_p \times \frac{\pi}{2} d_p^2 = \frac{3m_p}{\rho d_p}$$

where n_p is the number of particles, d_p is the Sauter mean particle size, m_p is the mass of the particles and ρ is the density of the particles

The theoretical particle coverage C is then given by the ratio of the maximum surface area that can be covered by the particles S_p and the total surface to be covered S_d :

$$(3-6) \quad C = \frac{S_p}{S_d} = \frac{m d_o}{2 \rho d_p V_{oil}}$$

From Equation (3-5) it can be derived that the average droplet diameter (d_o) should be inversely proportional to the mass (including size and number) of particles (m_p) which is in agreement with the other model.

All of these considerations are based on several assumptions including the contact angle, the particle size and uniformity as well as droplet size and uniformity and ideal monolayer packing of the particles. For complete surface coverage, the packing of the colloidal particles on the surface would need to have perfect hexagonal symmetry resulting in a packing density of $P \approx 0.74$ (Kepler conjecture). Topological constraints imposed by the curved surface, however, require a minimum of 12 five-fold disclinations, like the pentagons on a soccer ball (Bowick et al., 2000; Dinsmore et al., 2002) resulting in an even lesser packing density. Similar considerations lead Yusoff & Murray (2011) to use random packing for their model. They come to the conclusion that predicted versus experimentally determined oil droplet size do not correlate well. Further, for a close packing the colloidal particles need to aggregate which leads to multilayers of particles. The interested reader is referred to Bergström (2006) who reviews in detail the structure and formation of particle clusters and monolayers at liquid interfaces. In practice it is common to make experimental runs with increasing particle concentrations or constant particle concentration and increasing phase volume (Frelichowska et al., 2010; Juarez & Whitby, 2012; French et al., 2015).

The above mentioned model only applies for perfectly spherical particles but packing density also depends on particle shape. The maximum possible particle density decreases with decreasing aspect ratio of the particles. However, successful stabilization with particles other than spherical particles is possible. E.g. Alargova et al. (2004) and Tzoumaki et al. (2011) demonstrated Pickering stabilization with rod-like particles. Bon & Colver (2007) stabilized emulsions with disc-like Laponite particles.

While the functionality of surfactant stabilized emulsions heavily depends on the ability of the surfactant to reduce the interfacial tension, this is not the case for emulsions stabilized by solid particles (Lopetinsky et al., 2006). Even though Zargartalebi et al. 2013 reported that silanized silica particle reduce the interfacial tension of kerosene and water to half its original value, the majority of reports document no or only little interfacial activity for particles at the oil-water interfaces (Brian & Chen, 1987; Aveyard et al., 2000; Vignati et al., 2003; Wang et al., 2004; Ghouchi et al., 2007; Ravera et al., 2008; Whitby et al., 2008; Drelich et al., 2010 and Hu et al., 2015). Perino et al. (2013) even report higher interfacial tension in the presence of hydrophilic silica particles. Analytical methods used need to be considered in this context, since particles show sedimentation and thus several techniques are not reliable like e.g. the pendant drop technique, where particle sedimentation occurs.

The influence of pH and ions on Pickering stabilization is correlated with the conductivity of the continuous phase and therefore particle charge and state of aggregation (Binks & Whitby, 2005). Whitby et al. (2012) demonstrated the influence of salt concentration and its impact on zeta potential on the stabilization of Pickering emulsions. With decreasing zeta potential, the repulsive forces decrease, which allows the particles to mildly aggregate to form a dense layer around the oil drops (Reynaert et al., 2006). Flocculation is commonly found at a zeta potential of approximately zero which is also referred to as the isoelectric point (Köhler et al., 2010).

It was shown that silica is able to stabilize emulsions best when it is partly hydrophobized (Binks & Lumsdon, 2000; Persson et al., 2014). Emulsions with 1.5 % Aerosil R816, a hydrophobized silica, and 30 % dodecane were reported to be stable over several months even though the Sauter mean diameter was 25 μm (Whitby et al., 2012). Flocculation may transform the Pickering particles into larger aggregates thereby reducing their ability to reside at the oil-water interface (Binks & Lumsdon, 1999). In contrast, deflocculation may disrupt the network of particles and will thus reduce the energy required to laterally displace particles from the drop-drop contact region (Tambe & Sharma, 1994b). These considerations hold true for particles with low zeta potential or no charge. As mentioned above they form closely packed structures with the ability to stabilize emulsions. In addition, Nikolaides et al. (2002) describe attractive forces between like-charged particles at interfaces as the deformation causes capillary forces that attract neighboring particles to each other.

Even though many researchers emphasize the superior stability of Pickering systems, in most cases this only refers to droplet coalescence. However, a system stable to coalescence is not necessarily stable against creaming or sedimentation. Creaming is tolerated in some applications as unavoidable and the height of the cream layer is a characteristic parameter, which is used for comparing emulsion stability (Frith et al., 2008; Braisch et al., 2009; Morishita & Kawaguchi, 2009; French et al., 2015; Lee et al., 2014). However, creaming or sedimentation is in general not acceptable for a food product. Stability issues may also arise from a change of the composition of the surrounding medium. Frequently, food compounds are produced, which are incorporated by another manufacturer in specific foods. One example is dilution of aroma concentrates or clouding agents in non-alcoholic beverages. The particle interactions change upon dilution because the surrounding of the droplets change (Mishchuk et al., 2004). So far, no publication focused on the dilution of Pickering emulsions, but several authors (Binks & Lumsdon, 2001; Köhler et al., 2010; Varka et al., 2010 and Arditty et al., 2005) reported aggregation or flocculation when diluting samples for oil droplet size analysis by laser diffraction. Possible applications must be reviewed carefully considering stability during all steps of processing and in all dimensions. To improve stability, one may combine particles with surfactants (Tambe et al., 1995) which will be discussed in the following chapter.

3.2.3. Surfactants as co-stabilizers

The primary role of co-stabilizers is to control the wettability of the particles towards a 90° contact angle for maximum particle adsorption (Midmore, 1999; Mendoza et al., 2014). Low molecular weight surfactants also reduce the initial interfacial tension for better droplet breakup upon homogenization (Pichot et al. 2009; French et al., 2015). In the case of hydrophilic silica it was demonstrated, that a cationic surfactant (e.g. hexadecyltrimethylammonium bromide, CTAB) absorbs to the particle surface and renders them more hydrophobic. Resulting oil in water emulsions were most stable when particles had little to no charge and were most flocculated (Midmore, 1998; Binks & Whitby, 2005; Ravera et al., 2006; Lan et al., 2007; Binks & Rodrigues, 2007a; Sugita et al., 2008; Limage et al., 2010). As well, the performance of positively charged silica particles could be optimized by addition of anionic surfactant (Binks & Rodrigues 2007b; Whitby et al., 2009) or non-ionic surfactant (Nesterenko et al., 2014). The

stabilization effect is usually linked to the surfactant concentration (Binks & Rodrigues, 2007a; Santini et al., 2014). Above a critical surfactant concentration desorption of particles from the interface may be observed (Lan et al., 2007).

A question linked to the combined use of particles and surfactants is whether they compete at the interface (Drelich et al., 2010). In this context it is noteworthy that special attention should be given to the mixing protocol. Binks et al. (2007) highlight the difference whether particles are added to a surfactant stabilized emulsion, surfactant is added to a particle stabilized emulsion or both are added simultaneously and conclude that only the latter approach is successful.

Hu et al. (2015) demonstrated with confocal laser scanning micrographs of fluorescent cellulose nanocrystals that addition of a cationic surfactant before homogenization prevents surface occupation by particles with increasing surfactant concentration. Drelich et al. (2010) for example observed a slow destabilization of an initially stable particle stabilized emulsion upon addition of surfactant. Whitby et al. (2009) prepared stable oil-in-water emulsions with partially hydrophobized silica and diluted them into sodium dodecyl sulfate solutions. Above the critical micelle concentration the emulsions creamed and flocculated rapidly indicating that the surfactant displaces particles from the interface. Pichot et al. (2010) found lipophilic silica removed from the interface by polysorbate with increasing concentration whereas this was not the case for lecithin. Guzmán et al. (2012) on the other hand found palmitic acid removed from the interface because of adsorption on the silica particle surface. Ghouchi al. (2007) emphasize that stability is governed by initial particle location for emulsions composed of hydrophilic silica and lecithin or oleylamine as co-surfactant. Coalescence was greatly reduced when silica was incorporated through the oil phase. It becomes obvious that co-stabilized systems are as well strongly influenced if not driven by the mixing protocol.

This chapter highlights the importance of electrostatic interactions for successful stabilization. Emulsions usually were most stable around zero charge. On one hand, by using a co-surfactant the surfactant free character is lost, which was one of the drivers for the food industry. On the other hand this is a suitable strategy to bring particles to the interface and tailor desirable properties. The surfactant level in such systems would still be greatly reduced.

3.3. Food grade approaches

Since silica is only approved as anti-caking additive (E551) other materials are needed for the food industry, where many products are emulsion based (Guzey & McClemetns, 2006; Atarés et al., 2012). As mentioned above, the surfactant-free character of Pickering emulsions is very intriguing, since the food industry is highly interested in food-grade approaches for clean label products (Green et al., 2013). Consequently, extensive research has been done to investigate the possibilities of food grade approaches to Pickering emulsions. A comprehensive overview of particles used and formulations in food grade applications is provided in Rayner (2015) and Tavernier et al. (2016). Apart from a few specific studies on flavonoids, phytosterols, lactoferrin, shellac, cellulose or chitin nanocrystals (Campbell et al., 2008; Habibi et al., 2010; Kargar et al., 2012; Atarés et al., 2012; Dhar et al., 2012; Tzoumaki et al., 2011; Luo et al., 2011, 2012; Zoppe et al., 2012; Shimoni et al., 2013; Capron & Cathala, 2013; Liu & Tang, 2014; Salas et al., 2014), main focus in the last decade was to derive stabilizing particles from one of the three major food constituents protein, starch or fat. The latter are reviewed in more detail below.

3.3.1. Protein

Proteins are composed of amino acids with different side chains and therefore exhibit an amphiphilic character (Hoffmann & Reger, 2014). Particularly, dairy and soy proteins are well known for their emulsifying properties (Dickinson & Parkinson, 2004). Proteins aggregates dissociate and unfold upon adsorption at an interface, (Gao et al., 2013). Furthermore the molecular structure is sensitive to heat and pH (Leal-Calderon et al., 2007). This is why Dickinson (2012a) stated, that proteins per se cannot be considered as Pickering particles. As a consequence researchers tried to strengthen the structure of proteins for example by cross-linking (Dhayal et al., 2014; Wu et al., 2015) or heat treatment (Liu & Tang, 2013) to inhibit unfolding at the interface. The heat treatment leads to aggregation and formation of a gel-like structure. De Folter et al. (2012) prepared emulsions with zein, an insoluble corn protein with high hydrophobic acid content. Gao et al. (2014) prepared zein particles by ultrasonification with stearic acid, both considering zein as Pickering particles. The emulsions were stable under very defined pH conditions, which limits their usage (De Folter et al., 2012) and stability was mainly achieved by gel formation of excess protein in the continuous phase (Gao et al., 2014). Bressy et

al. (2003) came to the conclusion that sodium caseinate forms soft elastic shells around the stabilized oil droplets rather than single particles. Another limiting factor is that protein stabilized droplets may behave as sticky droplets (Hoffmann & Reger, 2014). This implies that droplets, even though they may not show coalescence, aggregate and therefore show creaming or sedimentation. Consequently only emulsions with very high internal phase volumes (≥ 0.6) may be stabilized by some proteins, which is for example the case for soy protein aggregates (Liu & Tang (2013).

Another approach to overcome the unfolding of the protein are recently for emulsion stabilization discovered protein microgels, which actually make use of the deformability of the protein structure as well as the pH- and thermo-responsiveness (Destribats et al., 2011; Rayner, 2015). Microgel particles in general are defined as a cross-linked polymer which is swollen by a good solvent (Saunders & Vincent, 1999) adopting a “fried egg-like” structure at the interface (Destribats et al., 2011). This structure is a colloidal entity with definite particle-like features, but without the solid-like character of a typical Pickering stabilizer (Dickinson, 2015). Food grade approaches are swollen and heat treated starch granules, inulin (Rayner, 2015) or other hydrocolloids like alginate or carrageenan (Dickinson 2015), but most often whey protein or protein-pectin complexes are used. A major advantage of whey protein – compared to other polymers used for microgels – is that it does not need cross-linking agents (Schmitt et al., 2010). These microgel particles are produced by polymerization of activated monomers in the presence of a specific solvent or reaction limiting secondary polymers (Schmitt et al., 2010). The stabilizing mechanism of microgels is strongly influenced by particle charges, the interfacial packing and the viscoelastic properties of the film (Destribats et al., 2011). It could be shown that microgels are susceptible towards flocculation, though resistance against coalescence is exceptional (Destribats et al., 2014). The charge of proteins depending on the dispersion medium determines the packing density and therefore the tendency to flocculation and bridging (Schmitt et al., 2010; Zimmerer & Jones, 2014). Richtering (2012) states that microgels as soft, porous particles are significantly different from classical rigid colloidal stabilizers in Pickering emulsions and suggests avoiding the term Pickering emulsion when swollen microgels are employed. While they may well work for encapsulation and controlled release, it remains to be seen whether they can serve as novel Pickering-type emulsifier.

Recently, extensive research focused on a newly discovered class of proteins: hydrophobins. Hydrophobins are relatively small proteins expressed by filamentous fungi. They consist of 100 ± 25 amino acids with a distinctive eight cysteine motif forming four disulfide bonds (Blijdenstein et al., 2010). The characteristic disulfide bonds result in a tight core, which prevents hydrophobins from unfolding at the interface (Blijdenstein et al., 2010) which is why they are classified as Pickering particles. Hydrophobins may adhere to any surface due to their amphiphilic character and therefore invert the hydrophobicity of this surface (Lumsdon et al. 2005; Sunde et al. 2008, Zampieri et al. 2010). In nature they serve as coatings around spores to facilitate their adhesion on surfaces or to colonize hosts (Grünbacher et al., 2014). On one hand they prevent desiccation on the other hand they prevent air channels in fruiting bodies from getting blocked by rain water (Wösten, 2001).

The amphiphilic character makes hydrophobins highly surface active and contributes to their self-assembling properties (Cheung, 2012; Green et al., 2013). Depending on the concentration hydrophobins exist as mono-, di- or tetramers. They quickly adsorb to the interface where they form strong elastic and dynamic films (Wohlleben et al., 2010). Thus, hydrophobins are comparable to so-called Janus particles, which are compartmentalized colloids with two different sides of chemistry or polarity (Walther & Müller, 2008). Hydrophobins may be classified into two groups based on differences in solubility and stability: class I and class II. Class I hydrophobins are highly water insoluble, while class II hydrophobins show better dispersibility in an aqueous environment. Class I hydrophobins resist 2 % sodium dodecyl sulfate (SDS) at 100 °C and can only be dissociated by trifluoroacetic acid, class II hydrophobins dissociate in 60 % ethanol and 2 % SDS (Vocht et al., 1998; Askolin et al., 2006; Stanimirova et al., 2014). Class I hydrophobins lower the air/water surface tension from 72 mN/m to 24 mN/m at concentrations of 50 µg/L, making them the most surface-active molecules known (Wösten & Vocht, 2000). Upon self-assembly at an interface they form a visible, light-reflecting membrane, which resists washes with water (Linder, 2009; Wösten, 2001; Zhang et al., 2011; Lienemann et al., 2015). This film solidifies over time and is irreversibly adsorbed (Alexandrov et al., 2012). On a microscopic scale a porous structure becomes visible which can be picked up as a discrete film (Wösten, 2001). The stability of hydrophobin membranes and their particle like character may be demonstrated upon compression of the film where the compressed layer shows wrinkles (Blijdenstein et al., 2010, Knoche & Kierfeld, 2015). Because of their unique properties

hydropobins are investigated in the context of a wide range of possible applications. In medicine they are studied for their possibility to stabilize emulsions with fluoruous fluids, as fusion proteins to encourage the binding of human fibroblasts on implant surfaces and to prevent bacterial growth on catheter surfaces (Scholtmijer et al., 2001; Milani et al., 2013). A similar approach is the use in biosensor applications to immobilize molecules or enzymes on surfaces (Wang et al., 2010). The grid-like membrane may also be used for membrane ultrafiltration or for chiral separation (Oude Vrielink et al., 2015). Applications in the field of home and personal care are eco-friendly dirt repellent, antifouling coatings for windows, cars and ships, eco-friendly surfactant for laundry detergents and heat protecting hair care products (Hektor & Scholtmijer, 2005; Wohlleben et al., 2010). BASF patented hydro-phobins as an additive to glue in bookbinding with improved flexibility (Baus et al., 2009).

Widely used in research is H Star protein B (HPB), which belongs to class I hydrophobins. It is soluble up to 5 g/100 mL at pH 7.54 and lowers the interfacial tension of the decane/water interface from 50 mN/m to 15 mN/m (Reger et al., 2011a). Reger & Hoffmann, 2012 and Reger et al. (2011a, 2011b, 2012) demonstrated that emulsions with HPB alone are not stable against creaming, if the phase volume of the dispersed phase is below 65 %. Addition of bohemite or laponite clay allows to minimize the phase volume down to 30 %. Furthermore, the authors verified a 3D network and bridging between drops upon drying of the emulsion. The application is therefore again limited to very viscous systems, which must not undergo dilution upon final preparation. Other researchers report extraordinary foam stability of so called air-filled emulsions, even from just shaking the sample (Cox et al., 2009; Tchuenbou-Magaia et al., 2011; Blijdenstein et al., 2010; Basheva et al., 2011). It could be shown that hydrophobins effectively stop disproportionation in foams (Blijdenstein et al., 2010). The use of hydrophobins as a foaming agent for aerated and aerated frozen food as fat replacer has been patented e.g. for ice cream, sorbets and low-fat whipping cream (Tchuenbou-Magaia et al., 2009; Cox et al., 2009; Baus et al., 2009; Aumaitre et al., 2009; Bot et al., 2013; Cox et al., 2014). Thus, on one hand hydrophobins offer new possibilities in aerated systems like milk shakes, mousses and creams, but on the other hand they have been identified as a major factor responsible for gushing in beer. As a consequence use of hydrophobins cannot be recommended for carbonated beverages (Khalesi et al., 2012; Shokribousjein et al., 2015). From an industrial perspective, the rather long time-scale required for assembling at interfaces along with the partly low solubility of

hydrophobins raises concerns with respect to processing of these proteins on a large scale (Tchuenbou-Magaia et al., 2009; Green et al., 2013). Furthermore the high affinity for surfaces taken together with their ability to form biofilms may cause hygienic problems (Rieder et al., 2011).

In summary it becomes obvious that proteins itself might not easily be utilized for Pickering stabilization. Their susceptibility towards pH and their tendency to unfold, makes structural modification necessary. The range of applications seems to be limited to systems with high internal phase volume and high viscosity, where particle aggregation is part of the physical stabilization or at least not disadvantageous.

Microgels show as well a promising development to overcome the structural changes at the interface. Nevertheless, possible applications are still limited to their susceptibility towards external conditions but will allow specific products. In contrast this property is highly intriguing for encapsulation and stimulus-responsive release.

Hydrophobins exhibit the potential for a wider range of dispersed systems. However, future research should focus on challenges related to large scale industrial processing.

3.3.2. Starch

The use of starch granules as Pickering particles bears several advantages. Starch is considered as a natural ingredient, is abundant in nature, renewable, biodegradable, thus sustainable, and inexpensive (Timgren et al., 2011; Dufresne, 2014). Timgren et al. (2013) and Li et al. (2013) compared the suitability of starch granules as Pickering particles from different botanical such as quinoa, rice, maize; waxy varieties of rice, maize, and barley; and high-amylose maize, wheat, potato. The authors reported that Quinoa starch exhibits the smallest granules with a volume weighted mean diameter of 2 μm . With particles of that size, oil droplets in emulsions could be stabilized at a mean oil droplet size of approximately 200 μm . Since native starch is not hydrophobic, attempts have been made to improve the wetting properties by partial hydrophobization with octenyl succinic anhydride (OSA) (Nilsson & Bergenståhl, 2007; Rayner et al., 2012b; Timgren et al., 2013; Miao et al., 2014). For commercially available OSA-starch this is an established procedure where esterification of depolymerized or hydrolyzed starch takes

place under alkaline conditions (Bao et al., 2003). In this commercially available type of OSA-modified starch, due to depolymerization and hydrolysis starch granules are disintegrated and the stabilization is therefore not Pickering-type stabilization. For Pickering purposes only the surface of intact starch granules is modified. However, the processing may cause partial breakdown of the starch granule. The smallest reported oil droplet size for emulsions with unmodified quinoa starch granules was 320 μm (Timgren et al., 2013) using 100 mg starch per ml oil and 50 % oil load. With OSA- modified quinoa starch an average oil droplet size of 27 μm could be achieved using 214 mg per ml at 56 % oil content (Marku et al., 2012).

Another approach to use starch as Pickering particle is based on the use of starch nanocrystals ranging from 10 – 300 nm which allow to generate emulsions smaller than 1 μm . Dispersions of starch nanocrystals were studied by Wei et al. (2014) and show sufficiently low zeta potential at low pH. Tan et al. (2012) created hydrophobized starch nanospheres by modification with acetic aldehyde and phthalic anhydride. Emulsions prepared using these nanospheres were only stable at a tricaprylin phase volume of 50 % or higher (Tan et al., 2014). Song et al. (2014) prepared OSA-modified particles from rice starch and prepared emulsions with 16 % – 75 % soybean oil. Emulsions were stable against coalescence, but not against creaming even at very high phase volume (Song et al., 2015).

Several questions remain to be clarified to draw a final conclusion on the suitability of starch-based nanoparticles for food-grade Pickering applications. Yusoff et al. (2011) express concern whether OSA-modified starch nanocrystals can still be considered particles. They found surface tension measurements for cryo-milled and homogenized OSA-modified starch dispersions decreasing to values typical for low molecular weight surfactants indicating that the processing lead to a disintegration of the granules. The milling process has the same effect like a heating step and will lead to particle disintegration and increase in amorphous structures (Belitz et al., 2008). Starch particles may disintegrate and gel at the interface as observed by Marefati et al. (2013) and Sjöö et al. (2015) who use this effect to improve freeze-thaw stability and barrier properties. All of these findings question, whether starch can be considered as Pickering particle. On the other hand Timgren et al. (2011) could demonstrate crystalline regions by polarized light in starch granules fused together at the interface by heat treatment. For starch nanoparticles confocal laser scanning microscopy images give prove for crystalline structures (Tan et al., 2012). The above mentioned articles demonstrate that starch performed best when slightly hydrophobized and

starch nanocrystals exhibit highest crystallinity after acid hydrolysis. From a regulatory point of view both treatments result in a modified starch. This leads to the second question, whether these particles are still food grade. Acid modification is approved for certain acids by the United States Food and Drug Administration (FDA) and the European Union (EU) (food additive E1404). OSA modification of starch up to 3 % is as well approved by (FDA) and the (EU) (food additive E1050). It is not permitted in other countries as for example India. The conversion of starch with succinic anhydride was first reported in 1953 (Tegge, 2004) and is already widely applied in the food industry. Therefore use of modified starch as emulsifier is per se not a new approach and would not deliver a solution to satisfy the food industry's demand of more label friendly ingredients.

3.3.3. Fat

Rousseau extensively reviewed the role of fat crystals in food emulsions (Rousseau 2000; 2013). He concludes that fat crystals can either stabilize or destabilize an emulsion depending on the localization. Crystals in the continuous phase may adsorb to an interface and add to the stabilization. In contrast, crystals in the dispersed phase may protrude through the interface and lead to droplet aggregation. The first proof of successful Pickering stabilization with fat particles was demonstrated with tristearin (SSS) by Lucassen-Reynders and van den Tempel in 1963 (Lucassen-Reynders & van den Tempel, 1963). It is well demonstrated that fat crystals partially or wholly stabilize oil continuous emulsions as for example butter, margarines and low fat spreads (Juriaanse & Heertje, 1988; Bergenstahl & Aalander, 1997; Rousseau & Gosh, 2009; Gosh et al., 2011). Several studies deal with the optimization of the crystallization or the effect other present emulsifiers impart on the stability of water-in-oil emulsions (Rousseau, 2000; Coupland, 2002; Gosh & Rousseau, 2011; Douaire et al., 2014). Therefore it is evident to see whether oil in water emulsions can as well be stabilized by fat particles. Paunov et al. (2007) demonstrated that unmodified fat crystal particles of hardened rape seed oil were only able to stabilize water-in-oil emulsions. For oil-in-water emulsions a combination of carbonate particles coated with stearic acid was successful. This resembles the findings for starch particles which only gain the desired properties via modifications.

Recently, there has been growing interest in creating so-called solid lipid particles (SLP) for pharmaceutical applications (Svilenov & Tzachev, 2014) or oil in water emulsions with a solid hydrophobic shell stabilizing the dispersed phase (Salminen et al., 2013). These systems are usually fat-in-water dispersions prepared from high melting emulsifiers (HME) such as phospholipids. The aim is to create a dense solid structure through crystallization of the lipid phase starting from the interface. The resulting structure facilitates encapsulation and protection of lipophilic active ingredients and is thus also interesting for the food industry. Kurukij et al. (2013) demonstrated the successful preparation of emulsions stabilized by sodium stearyl lactylate (SSL) which has a melting point of 49°C. Via differential scanning calorimetry (DSC) it was demonstrated that SSL was in solid state at the interface at room temperature. Salminen et al. (2013) were able to produce dispersions stabilized by high melting phospholipids (lecithin). However, both emulsifiers need to be labelled as a food additive and thus, despite the promising results, there might be a challenge in finding a suitable food grade ingredient which will be label friendly. Another interesting fact is that upon crystallization fats usually undergo an increase in density due to the closer packing in the crystal lattice. This may positively affect the buoyancy of the oil phase and thus the stability of the emulsion against creaming. Since creaming is a very prominent issue, this makes fat particles intriguing candidates for particle stabilized emulsions.

It can be concluded that fat particles are outstanding stabilizers in water-in-oil food emulsions. Recent results for SLPs, makes fat particles interesting candidates as well for oil-in-water systems, especially under the presumption that they additionally can improve stability by increasing the density of the oil phase. Further research should focus on finding suitable and label-friendly solutions.

3.4. Concluding remarks on Pickering emulsions in food-related applications

As previously mentioned dispersed systems are very common among foodstuffs (Guzey & McClements, 2006). Products such as soft drinks, milk, coffee whitener, cream, whipped cream, ice cream, salad dressings, mayonnaise, soups, sauces, dips, butter and margarine are only few examples of emulsion-based systems in the food sector (McClements, 1999; Guzey & McClements, 2006; Atarés et al., 2012). As a consequence, there are tremendous differences in

physical structure and consumer-related requirements e.g. towards specific properties like texture, shelf life and so forth.

One of the most important factors for successful Pickering stabilization is minimization of the attractive forces between the particles to form a dense layer at the interface. These may be adjusted as could be demonstrated for starch nanocrystals (Murray et al., 2011; Gao et al., 2014; Hu et al., 2015) and silica particles (Midmore, 1998; Binks et al., 2007; Ghouchi et al., 2007; Maas et al., 2010; Whitby et al., 2012; Sadeghpour et al., 2013; Santini et al., 2014) upon the addition of non-ionic surfactants and fatty acids like oleic or palmitic acid.

Lack of electrostatic repulsion inevitably leads to aggregation of the stabilized dispersed phase. Systems exhibit extraordinary stability towards coalescence, but creaming or sedimentation can only be avoided when a 3D network in the continuous phase is formed. Another important factor is the dependency of the stability from the phase volume. Only systems with high internal phase volume can successfully be stabilized against creaming. Lagaly et al. (1997) pointed out that the network formation is linked to the phase volume. Typically focus on research of Pickering systems is the stability against coalescence whereas for a stable food application stability against coalescence and creaming must be guaranteed. The need for aggregation to yield stability is as well the limiting factor for the minimum phase volume. For a wide range of applications it is sensational to be able to stabilize high internal phase volumes by a small amount of particles. The same applies for paste like water-in-oil emulsions like confectionary creams with high viscosity. Binks & Lumsdon (2001) prepared emulsions, in which the dispersed oil phase showed sedimentation because the particle adsorption altered the overall density of the dispersed phase. Therefore, high density of Pickering particles could possibly be used as well to adjust density differences between the phases and thus increase physical stability of the system. Rayner et al. (2012a) demonstrated that sedimentation increased with increasing starch particle concentration. Large density differences e.g. occur in non-alcoholic beverages. The continuous phases is high in sugar and shows a rather high density, while aroma oils have typically a low density especially citrus oils and need weighting agents for density adjustment.

Pickering particles may also be used to alter the optical properties of a dispersed system. Velez (2000) stated that closely packed crystals display a number of potentially usable characteristics such as light diffraction. Due to closer packing of molecules in highly ordered structures and the

birefringence effects caused by different spacing between the atoms, crystalline structures inhibit stronger light scattering properties than amorphous substances (McClements, 1999). As a consequence the optical properties of an emulsion are not only affected by the oil phase, but also by the composition of the interface. Particles may be used to adjust difference in the refractive index between dispersed and continuous phase. This effect is highly relevant for applications where turbidity is a desired property, like in beverage emulsions. Typical values for the refractive index of polysaccharides are about 1.46 (Kasarova et al., 2007), which is lower than the refractive index of most of the oil phases used for beverage emulsions. Because of their crystal structure fats should exhibit a higher refractive index than oil and might be a promising choice in this context. On the other hand one has to keep in mind that aggregation and network formation is detrimental for dilute systems like beverages with near water viscosity or intermediate products, which undergo further dilution before consumption. Since the initial particle structure will be destroyed during the process, those emulsions will be prone to creaming.

Superior stability of Pickering stabilized foams offers good opportunities for application and fat reduction in aerated creams, ice-cream, desserts and shakes. In own studies it was observed that emulsions immediately after preparation contain a high proportion of occluded air and therefore represent very fine foams. This foam stays stable for several weeks. In this state it appears that there is a single expanded network of particle stabilized bubbles and droplets as described by others (Juarez & Whitby, 2012), which even measure a yield stress (Braisch et al., 2009; Chen et al., 2012). Upon mechanical agitation foam settles and emulsions did not show yield stress or any shear thinning behavior. Still the emulsions did not separate in visually detectable phases for several months. Highly stable foams are desirable in a wide variety of applications, e.g. for milk froth of coffee drinks, milk shakes and ice cream. Very fine foams can probably add to the mouthfeel of fat reduced varieties of whipping creams, spray cream, cream cheese, mayonnaise, ice cream, sauces, vegetarian spreads or meat paste. As mentioned above, first patents using hydrophobins to create fat reduced foods have been filed. Fat replacement may also be achieved through reduction of the oil load by using double emulsions. Matos et al. (2013) stabilized double emulsions with OSA-modified Quinoa starch granules and recommend this system for fat replacement or as vehicle for hydrophilic nutrients.

Encapsulation and release of lipophilic ingredients via Pickering emulsions has also been investigated. Tikekar et al. (2013) demonstrated sustained release of curcumin over an extended

time period from silica stabilized emulsions. Recently, the feasibility for a food-grade approach could be demonstrated in a comparable study for OSA-modified Quinoa starch granules (Marefati et al., 2017). Kargar et al. (2011) and Zhao et al. (2015) prepared emulsions with enhanced oxidative barrier properties using silica and silica–polymer microparticles. Controlled release and improved encapsulation of lipophilic ingredients seem to be tunable, but to the best of the author’s knowledge no food grade approaches have been published yet.

In summary, this review of food grade Pickering approaches demonstrates the possibilities and limitations of particle stabilization in the food sector. With a proper choice of a particle, oil-in-water as well as water-in-oil emulsions can be achieved. For highly viscous products, products with a high internal phase volume and foams Pickering particles offer alternatives to commonly used surfactants. Pickering emulsions might be able to offer new approaches for fat reduction as well as encapsulation and sustained release of active ingredients. Nevertheless, a major part of successful systems have been achieved with silica or modified particles, which is not in line with the consumer demand for clean label, natural systems or not even food grade. However, the intriguing possibilities motivate and justify future research on the identification of new suitable ingredients, improvement of existing formulations and identification of new fields of application.

3.5. Acknowledgements

The authors would like to thank Gerhard Krammer and the Symrise AG for supporting this work.

4. Manuscript III: Solid fat content determination of dispersed lipids by time-domain NMR

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Accepted manuscript in *European Journal of Lipid Science and Technology* (27 January 2018)
<http://doi.wiley.com/10.1002/ejlt.201700132>

4.1. Abstract

Time domain nuclear magnetic resonance (TD-NMR) was used to determine the solid fat content (SFC) of dispersions stabilized by a high molecular weight hydrocolloid (gum arabic, GA) containing a high melting lipid (tristearin, SSS). Since the lipid was molten prior to homogenization for the preparation of the dispersion it recrystallizes during cooling and storage. It is known that the recrystallization in a disperse system differs from bulk lipids and TD-NMR could be a helpful tool to investigate the phenomenon. SFC measurement by TD-NMR is well established and widely used in food science for oils, spreads and seeds. In the case of dispersions, however, the situation is more demanding due to the quasi-continuous phase. A suitable TD-NMR method has to account for that and can be realized when combining longitudinal and transverse relaxation properties to separate the signal contribution of the quasi-continuous phase and the solid lipid and liquid lipid dispersed phase. Signal from water and emulsifier was eliminated by magnetization inversion with an initial 180° pulse and a subsequent dedicated inversion delay. The emulsifier showed a longitudinal relaxation similar to the aqueous phase. With a carefully chosen inversion delay, both signal contributions of water and emulsifier could be suppressed, and a quantitative determination of the SFC was possible via dedicated data processing.

Keywords: food emulsion, solid lipid particle, solid fat content, TD-NMR, high water content

4.2. Practical applications

The method can be applied in characterization, stability tests and quality control of complex food emulsions and SLPs, which are recently under development as drug carrier systems.

4.3. Introduction

SFC determination in bulk fats and oils with low resolution or TD-NMR is well established and standard procedures are available DIN EN ISO 8292-1; DIN EN ISO 8292-2; AOCS Cd 16b-93 and AOCS Cd 16-81(The American Oil Chemists' Society, Reapproved 2009a, Reapproved

2009b), and IUPAC 2.150 (International Union of Pure and Applied Chemistry, 1992). However, for SFC determination in food emulsions or solid lipid particles (SLP) problems arise isolating the dispersed (droplet) phase signal from the quasi-continuous phase (Johns, 2009). In addition, the transverse water relaxation is strongly affected by the food network structure (Gianferri et al., 2007), which complicates a direct SFC-like measurement combined with free induction decay (FID) deconvolution.

It is known that the crystallization of fats in dispersion differs from the crystallization in bulk (Coupland, 2002; Douaire et al., 2014). This is due to the fact that a seed crystal would be needed in each oil droplet in order to start crystallization (McClements, 2012b). Fat in small droplets crystallizes slower than fat in large droplets (Dickinson et al., 1993) and the melting temperature decreases with decreasing droplet size (Unruh et al., 1999). Depending on its molecular structure the emulsifier may either impede crystallization or act as template at the interface (Cerqueira et al., 2006; Bunjes & Koch, 2005). McClements et al. (1993) reported that an emulsifier with hydrocarbon tail similar to the structure of the fat promotes crystallization. On the other hand emulsifiers structurally different to lipids may delay nucleation and inhibit growth (Cerqueira et al., 2006). Therefore, it would prove particularly valuable to have techniques which enable to quantify the SFC and to observe phase transitions in single, fine droplets (Coupland, 2002). As has been reviewed by Douaire et al. (2014) different approaches are currently used or under development to characterize the physical structure of lipids in dispersed systems including DSC, X-ray diffraction (XRD), polarized light, microscopy and lately synchrotron radiation. While it is possible to get time-resolved information about the crystallization behavior and the lamellar structure, the mentioned techniques require sample preparation, tedious measurement and/or sophisticated equipment not readily available. Attempts have been made to calculate a crystallinity index from DSC results (Schubert & Müller-Goymann, 2005; Keck et al., 2014). The integration of a thermogram of an endothermic process is not only correlated to the melting enthalpy and to the mass of the sample but also to its heat capacity (Le Botlan & Ouguerram, 1997) which depends on the tempering process (Walker & Bosin, 1971). Since heat capacity differs for different polymorphic forms this seems not an appropriate for polymorphic lipids. There is no fast and reliable method to quantitatively describe the SFC in samples with finely dispersed fat droplets in representative quantities and suitable for routine quality control.

In this context a major advantage of TD-NMR is the nondestructive, representative sampling and the user friendly handling, once a method is set up (Voda & van Duynhoven, 2009; Duval et al., 2006). In NMR two main relaxation parameters are often explored: the longitudinal or spin-lattice relaxation with relaxation time T_1 describes the return of magnetization to thermal equilibrium after excitation by radio frequency. The transverse or spin-spin relaxation with relaxation time T_2 is an entropic process describing the decay of the transverse spin magnetization. Both parameters are known to depend on the substance under investigation. For more detailed background on NMR the interested reader is referred to (Renou et al., 2011; Hemminga, 1992; van Putte & van Den Enden, 1974).

The classic, established SFC determination by TD-NMR relies on the fact that the liquid and solid parts of the oil phase can be differentiated because transverse relaxation is usually much faster for solids than for liquids. The FID can be analyzed by an appropriate differentiation of the two components of liquid and solid lipid (Figure 4-1). Classically, correction factors take into account technical imperfections like probe dead time and B_0 inhomogeneity.

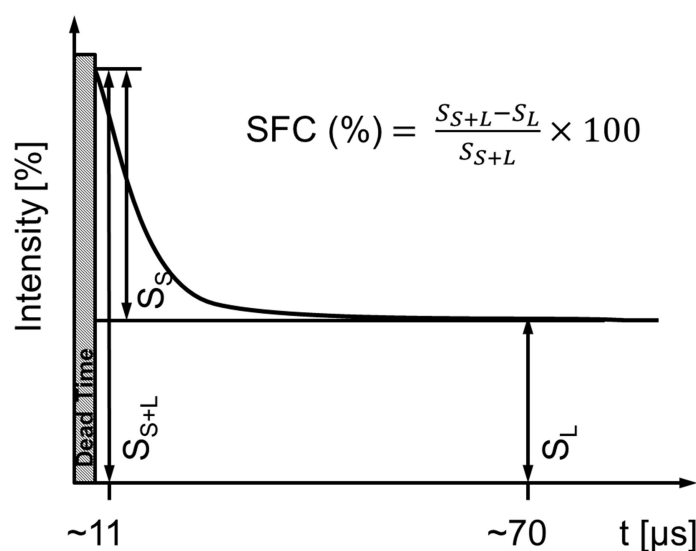


Figure 4-1: Principle of the classic SFC-measurement by TD-NMR: The FID contains components of liquid (SL) and solid substance (SS) which can be separated by their different transverse relaxation.

Generally, in foods, due to the complex composition, the longitudinal and transverse magnetization relaxation shows a complex behavior. Interpretation of these data is ambiguous (Gianferri et al., 2007). Depending on the chemical and structural composition, essentially two approaches are known to determine oil and water content in foods: Low water foods with water content up to 10 – 15 % can be analyzed by conventional TD-NMR methods based on transverse relaxation differences (Todt et al., 2006). Measuring a sample with high water content is challenging and is nowadays addressed by dedicated pulse sequences (Guthausen et al., 2004). These approaches often use chemometric data treatment, and therefore require calibration (Rudi et al., 2008). In addition, the main difference to the present samples is that the oil phase is dispersed in water and the degree of crystallinity of this dispersed oil phase is of interest. Therefore, the aim of the present paper was to demonstrate that the NMR signal of dispersions with high water content and a high molecular weight hydrocolloid as emulsifier, namely gum arabic, can be quantitatively analyzed for SFC by extension of the conventional SFC measurement principle by magnetization inversion followed by an appropriate recovery delay (Figure 4-2). The method was established using two samples with different SFC. One sample was an emulsion based on a medium chain triglyceride (MCT), which is completely liquid at room temperature. The other sample was made with tristearin (SSS), which in bulk is completely solid at room temperature (Bernreuther & Schimmel, 2011). The physical condition was proven by DSC measurements. To ensure that the emulsions were comparable, the droplet size distribution was kept constant.

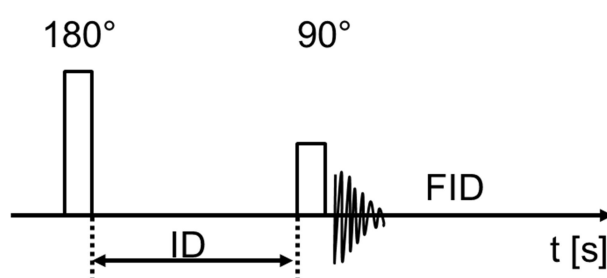


Figure 4-2: Pulse sequence for selective water suppression via longitudinal relaxation: FID: Free induction decay, ID: inversion delay.

4.4. Material and methods

The following materials were purchased or kindly provided by the suppliers: Gum arabic Senegal type (GA, Nexira GmbH, München, Germany), medium chain triglyceride (MCT) and tristearin (Cremer Oleo GmbH & Co. KG, Hamburg, Germany), potassium sorbate (Eastman Chemical Company, Kingsport, USA), and citric acid (Jungbunzlauer Suisse AG, Basel, Switzerland).

4.4.1. Preparation and characterization of the dispersions

The dispersions were prepared via hot high pressure homogenization method as described by Salminen et al. (2014) and consisted of water (79.5 %), emulsifier (13 %) and oil/fat (7 %) and preservatives (0.5 %). First, a solution of the emulsifier (ES) was prepared by mixing 17 % GA in filtered tap water at room temperature. Potassium sorbate and citric acid were added to prevent microbial growth. The solution was stirred at 2000 rpm using an IKA RW 20 digital (IKA®-Werke GmbH & Co. KG, Staufen, Germany) until the emulsifier completely dissolved. Temperature was set to 85 °C (10 °C above the melting point of SSS). The lipid phase was fully molten in a hot water bath at 90 °C. A coarse emulsion was prepared at 6000 rpm for 5 min with an Ultra-Turrax T50 with dispersion unit S50N (IKA®-Werke GmbH & Co. KG, Staufen, Germany). Subsequent homogenization was performed in three cycles with a laboratory table top model homogenizer NS1001L2K (GEA Niro Soavi S.p.A., Parma, Italy). Pressure was adjusted to achieve a similar droplet size with an $\bar{x}_{1,2} \approx 0.2 \mu\text{m}$ in both samples (25/5 MPa for MCT emulsion, 50/5 MPa for SSS dispersion). To prevent crystallization during homogenization the homogenizer was flushed with hot water (90 °C). Emulsions prepared with MCT oil were prepared at room temperature. Samples were immediately filled in NMR-tubes (see below) and stored at 20 °C left to crystallize for 40 h (according to DIN EN ISO 8292-2:2008 (2010)).

Droplet size was measured by laser diffraction using a Malvern Mastersizer 3000 equipped with a dispersion unit Hydro 2000S (both Malvern Instruments Ltd, Worcestershire, UK). For each sample five measurements were made using the following parameters: pump speed was set to 2000 rpm, measurement delay was 2 s, measurement time was 10 s, and 10,000 snaps were taken. For dispersions with different oil phase trials were undertaken to optimize the optical parameters. The instrument software provides two quality parameters of data fit. Optimization means that

similar and low values for the residuals and the weighted residuals should be achieved as these parameters reflect the correctness of the calculation of the distribution. For emulsions with MCT the refractive index (RI) is 1.45 and absorption is 0.001 for both blue and red laser. For SSS the RI is 1.52 and abs. is 0.001 for both blue and red laser. Laser diffraction measurement was undertaken and reported following ISO 13320:2009 (2009) and ISO 9276-2:2014-05 (2014).

DSC was used to determine the melting and crystallization behavior of SSS in bulk and in dispersion and to verify the liquid state of the MCT emulsion. The samples were analyzed with a 200 F3 Maia, Netzsch calorimeter using low pressure closed lid aluminum pans (both Erich Netzsch GmbH & Co. Holding KG, Selb, Germany). For reference, the melting points and the enthalpy of the raw materials were determined with 8 – 10 mg of sample. 22 – 27 mg sample was used for liquid dispersion. Air was used as reference. The samples were heated from 20 °C, respectively 4 °C to 100 °C with a heating rate of 5 °C/min and after 5 min at 100 °C cooled down to 0 °C with a cooling rate of 5 °C/min.

Time domain nuclear magnetic resonance (TD-NMR) was measured with a 20 MHz low resolution NMR “Bruker the minispec” mq20 with a H20-10-25(33)RVGX probe (Bruker Corporation, Rheinstetten, Germany). Data were acquired using “*the minispec Software*” V2.59Rev06/NT. T_1 and T_2 were measured for all bulk materials using standard inversion recovery and CPMG pulse sequences.

The SFC of the raw material was analyzed according to the indirect method (DIN EN ISO 8292-2:2008, 2010) since the melting temperature of HPF and SSS were out of range for the direct method. Analysis of the molten state was done at 90 °C, the SFC was determined at 20 °C. The manufacturers certificated standard set E1201050 was used to calibrate the instrument.

Data of the dispersions were acquired with the self-written customized ExpSpel script for an inversion recovery sequence shown in Figure 4-2. Special emphasis was put on storage of parameters and data in order to allow for the subsequent dedicated data analysis developed in this work. The acquisition and timing parameters were optimized using the water phase with dissolved preservatives as reference sample, since most of the signal will result from this phase (Table 4-1). The probe was tempered to 20 °C with a cryostat CP25 (Thermo Haake GmbH, Karlsruhe, Germany). Air was flushed through the probe to prevent condensation (Gribnau, 1992). A 1 cm spacer (Bruker) was inserted into the probe to position the sample tube in the

homogeneous region of the radio frequency (RF) coil. Immediately after preparation, 350 μ l ($\sim$ 1 cm filling level) of sample was pipetted into cylindrical glass inserts with 45 mm length and 7 mm outer $\varnothing$ which are placed in 10 mm outer $\varnothing$ NMR-tubes. Inserts were sealed with plastic paraffin film, test tubes were closed with caps (tubes, inserts and caps purchased from Bruker). Data analysis was executed in *JMP*® 13.0.0 (SAS Institute Inc., Cary, USA).

Table 4-1: Instrumental parameters of “the minispec” mq20 used for measurements

Parameter	Value
Detection Angle broad [°]	196
Detection Angle narrow [°]	200
Detection mode	magnitude
Band width	broad
90° Pulse [μ s]	2.5
180° Pulse [μ s]	5.1
Gain [dB]	75
Scans	40
Recycle Delay [s]	15
Pulse Attenuation [dB]	6

4.4.2. Procedure and parameters

Usually an established reference method is used to quantify the results (DIN EN ISO 8292-1:2010-08, 2010a; DIN EN ISO 8292-2:2008, 2010a; Green, 1996). Since there is no reference method to determine SFC in emulsions, this well-established approach was not applicable in this case as for example also for simultaneous water and fat determination in caramel mass (Rudi et al., 2008).

Ideally, a NMR probe does not show a background signal. In reality, some signal is present, and ringing down of the RF pulse is unavoidable. Since the method applied for the determination of the SFC in dispersions will be sensitive to this background signal, it was measured with an empty

test tube and revealed a pattern similar to a solid fat decay curve. This background signal was found to depend on the probe, i.e. it was specific for one of the instruments used. This background signal was found to depend on the probe, i.e. it was specific for one of the instruments used. It was also found to be highly reproducible and thus introducing systematic errors in the SFC determination which must be corrected for. Repeated measurements (20 repeats with and without test tube over several days) showed a very stable and reproducible signal background which therefore was regarded as a systematic, known error. In the analysis of the subsequent measurements the background signal average was subtracted in order to correct for the known systematic error.

The first step in method development was to measure T_1 and T_2 for all components of the samples to see whether the relaxation behavior is principally different so that a separation of the signal is possible. Transverse relaxation T_2 was measured via the CPMG pulse sequence (Günther, 2013;

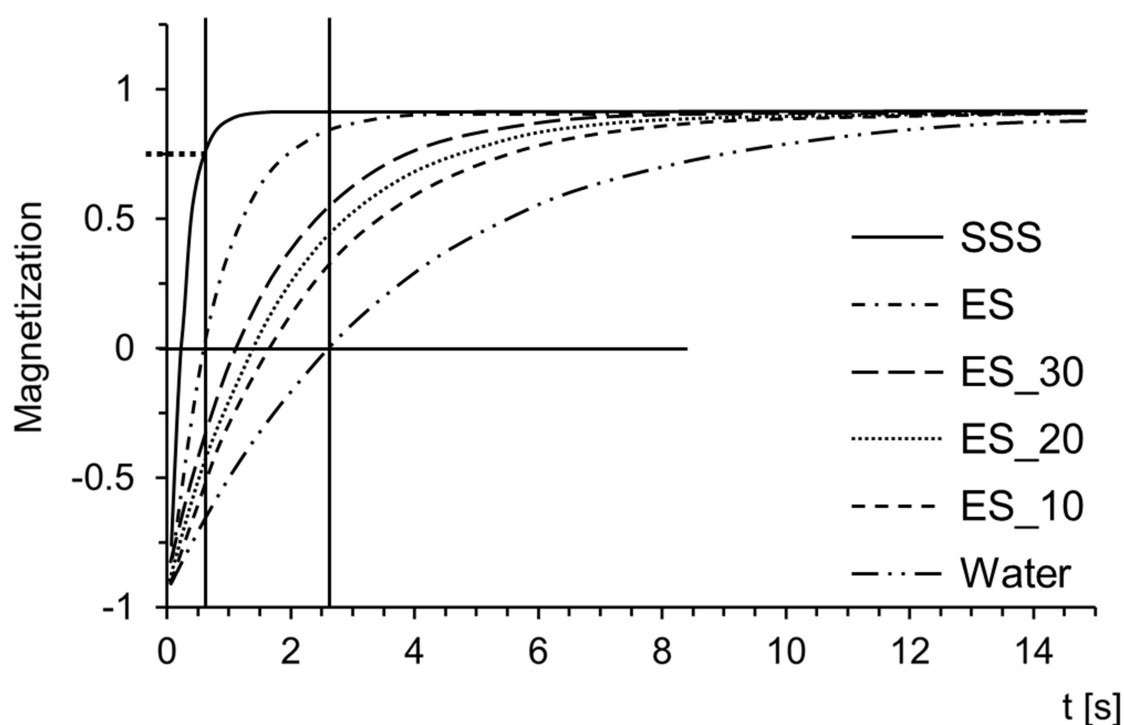


Figure 4-3: Simulation of magnetization inversion recovery for water (W), tristearin (SSS) and different concentrations of free emulsifier [100 % emulsifier slurry (ES) and 10 % (ES_10), 20 % (ES_20) or 30 % (ES_30) of original emulsifier concentration]. The zero-crossing is indicative for the chemical environment of the observed ^1H .

Kimmich, 1997). The transverse relaxation of the solid like fractions was estimated from the FID which is determined by T_2^* , a fact that is essential for the SFC determination. These reference measurements were performed at 40 °C. T_1 was measured by inversion recovery with the probe head tempered to 20°C as described above.

The chosen pulse sequence for SFC determination in an emulsion (Figure 4-2) exploits longitudinal and transverse relaxation. First a 180° pulse was applied to the sample, followed by a carefully optimized inversion delay (ID). The NMR signal is then recorded after a 90° pulse as an FID. Depending on T_1 the protons in different chemical environments will pass through a state of zero magnetization, a phenomenon which is exploited also in droplet size determination of O/W emulsions via TD-NMR (Goudappel et al., 2001). To separate water from fat and oil signals, the inversion point of water magnetization needed to be determined: During T_1 relaxation in an inversion recovery experiment the magnetization shows a zero-crossing (Figure 4-3), where the specific magnetization contribution to the measured signal is zero. Thus, the remaining magnetization of the target component can be exploited for further differentiation of signal contributions. To predict the possibility to separate the signals T_1 recovery curves were simulated according to Equation (4-1) with previously measured T_1 values taken from Table 4-2 for water, fat and ES.

$$(4-1) \quad M(t) = M_0 \left(1 - 2 \exp \left(\frac{-t}{T_1} \right) \right)$$

$$(4-2) \quad t_0 = T_1 \times \ln 2$$

The predicted times for zero-crossing of magnetization are displayed in Figure 4-3. Solving Equation (4-1) for t_0 leads to Equation (4-2). As the T_1 relaxation time for pure water was determined to be 2.59 s (Table 4-2), the corresponding value of t_0 is 1.80 s for water according to Equation (4-2). As it is known that slight changes of chemical composition and temperature influence the water T_1 relaxation considerably, a thorough optimization of ID has to be performed. Starting with an ID of $t_0 = 1$ s the ID for water with preservatives was scanned in small increments for determination of the signal minimum of the FID at $T = 20$ °C. The detected signal minimum was found at 1.7 s which would correspond to $T_1 = 2.45$ s, a common value for water relaxation at 20 MHz in the presence of salts and oxygen, for example. If the quasi-continuous phase behaves similar to water, the samples can be measured with ID = 1.7 s while neglecting

surface relaxation. Summarizing, the differences between calculated and measured T_1 are due to slightly different chemical composition and temperature, both parameters are well known in NMR to have a major impact on water T_1 relaxation.

Table 4-2: T_1 and T_2 for emulsion components [100 % emulsifier slurry (ES, see sample preparation); 30 % (ES_30), 20 % (ES_20) or 10 % (ES_10) of original emulsifier concentration; MCT = medium chain triglyceride; and SSS = tristearin] with an estimated instrumental accuracy for relaxation measurements of $\pm 10\%$; calculated t_{0calc} [$t_0 = T_1 * \ln 2$ (Equation (4-2))] and experimentally determined t_{0exp} ; solid fat content (SFC) measurement results with $n = 2$ repetitions.

Sample	T_1 [s]	T_2 [s]	t_{0calc} [s]	t_{0exp} [s]	SFC [%]
H ₂ O	2.59	n.a.	1.80	1.70	-
ES	0.68	0.32	0.47	0.42	-
ES 30	1.53	1.01	1.06	1.02	-
ES 20	1.75	1.33	1.21	1.19	-
ES 10	2.06	1.67	1.43	1.43	-
Gum arabic (powder)	0.08	0.016	0.06	-	-
MCT	0.17	0.16	0.12	-	0.09 / 0.07
SSS	0.17	0.0001	0.12	-	98.9 / 98.8

The relaxation of water molecules in protein and starch solutions is known to be affected by the macromolecular network, i.e. strong interactions and chemical exchange of water molecules with the protein and starch molecules (Ritota et al., 2008; Hansen et al., 2009; Lucas et al., 2005). This effect can also be expected for solutions of a large and highly branched hydrocolloid like gum arabic. To determine the influence of the emulsifier, the ES (17 % GA in water) and dilutions thereof (10 %, 20 % and 30 % ES) were screened for the zero-crossing time t_0 as well (Figure 4-3). The integrally measured T_1 depends on the emulsifier concentration as expected (Figure 4-4,

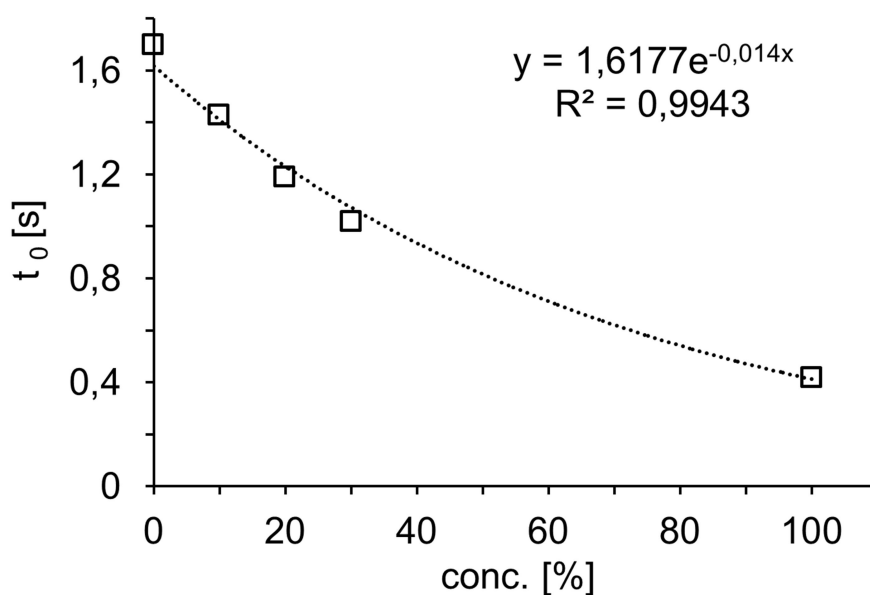


Figure 4-4: t_0 of water-emulsifier solutions: The integrally measured t_0 depends on the emulsifier concentration, with 100 %: concentration used for emulsion preparation and 0 %: preserved water phase.

Table 4-2) with t_0 decreasing with increasing emulsifier concentration. Subsequently, the dispersion samples were screened for signal minimum for the whole interval between t_0 for pure ES and pure water (0.42 – 1.7 s, see Table 4-2). At an ID of 0.5 s a good fit value was found at which the fast and slowly decaying components can be determined. Measuring the FID of dispersions with an ID of 1.7 s revealed a more Gaussian like decay rather than an exponential decay for the liquid components (Figure 4-5) which is due to the B_0 inhomogeneity of “the minispec” used for measurements. The Gaussian like shape becomes evident on large time scales of data acquisition (2 – 4 ms). The nearly time-independent behavior at small times < 1.8 ms opens up the possibility to determine the solid part of the sample very accurately – as the liquid part hardly changes in that timescale (Figure 4-5 insert) which is the basis for classic SFC determination. Therefore, the quantification of the solid oil and the liquid oil on the short time scale is relatively straight forward. The intercepts of the fast and slowly decaying parts were determined by extrapolation to $t = 0$, and consequently the SFC was calculated according to Equation (4-3):

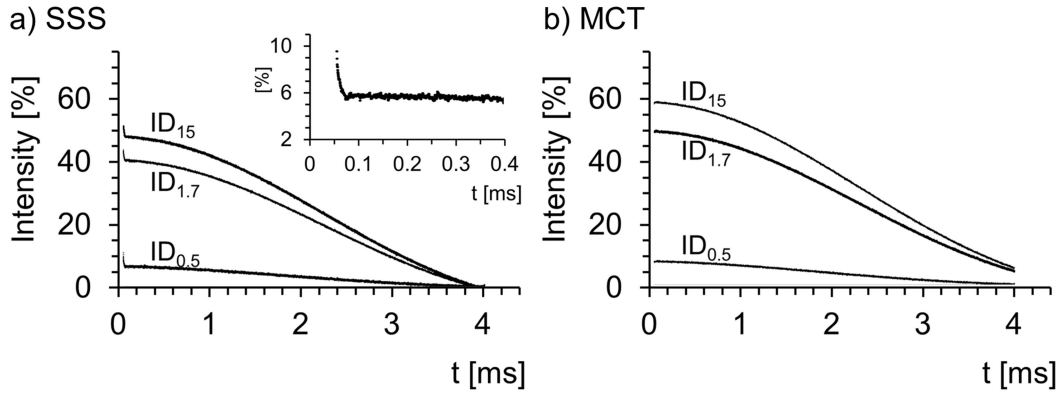


Figure 4-5: FIDs of a) tristearin (SSS) and b) medium chain triglyceride (MCT) dispersions. The amplitudes decrease with the inversion delay (ID), here $ID_{15} = 15$ s, $ID_{1.7} = 1.7$ s and $ID_{0.5} = 0.5$ s. Please note the different shapes at the very beginning of the FID which point directly to the different SFC values of MCT and SSS emulsions. Insert: Small time scale of SSS ($ID_{0.5}$)

$$(4-3) \quad SFC (\%) = \frac{I_{S+L} - I_L}{I_{S+L}} \times 100$$

where I_{S+L} is the intercept of the total signal and I_L is the intercept of the liquid signal.

Extrapolation of the solid regimen was done by linear fitting of the data between 0.008 – 0.023 ms. The decay of the liquid i.e. slowly relaxing components was fitted linear for the data between 0.097 – 1.6 ms. To test the reproducibility, 20 measurements with the same sample were performed.

4.5. Results and discussion

First, the results of the sample characterization are presented. Two samples with similar droplet size (volume surface weighted mean diameter $\bar{x}_{1,2} = 0.2 \mu\text{m}$) and the same volume of oil/fat were prepared. DSC thermograms (Figure 4-6) clearly show, that no melting (Figure 4-6a) or crystallization (Figure 4-6b) occurred for the MCT emulsion whereas for SSS in bulk and dispersion melting and crystallization occurred. The differences in peak height between the bulk lipid and the dispersion are due to the lower total fat content in dispersion (7 %, see sample preparation) compared to bulk lipid (100 %). The sample volume of the dispersion was more than twice compared to the bulk fat sample. In addition, the fat is not considered to be completely solidified in dispersion. The melting process is divided into two stages starting with one peak at

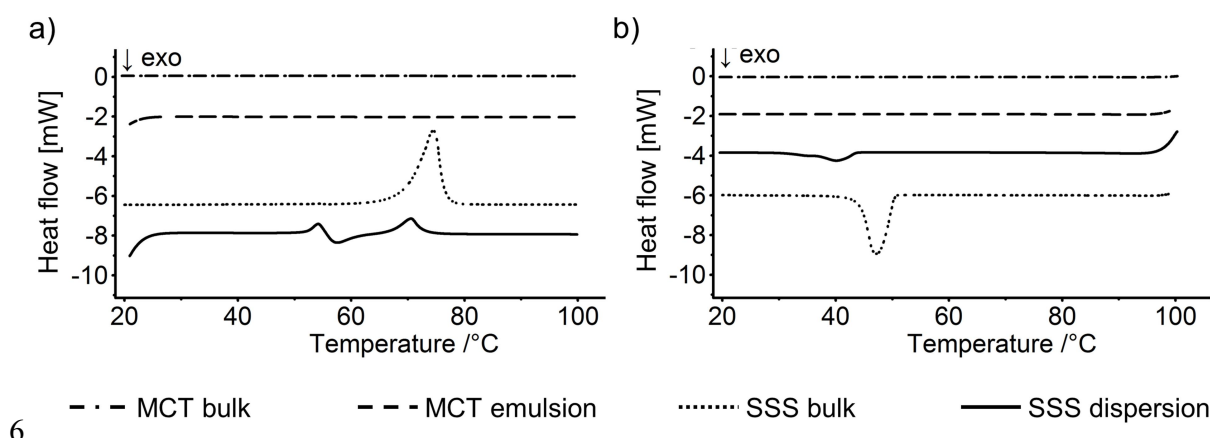


Figure 4-6: DSC heating (a) and melting (b) thermographs for medium chain triglyceride (MCT) and tristearin (SSS) as raw material in comparison to MCT and SSS in dispersion. Samples were heated from 20 °C to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min.

54 °C, which can be ascribed to α -polymorphs (Sato & Ueno, 2011) and another one at 70.6 °C belonging to β -polymorphs. It is well accepted that melting and crystallization behavior in the SSS-based dispersion significantly differs compared to the bulk fat. The melting temperature in the dispersed system is reduced by 4 °C compared to the bulk material (74.7 °C) as expected. Decreased melting temperature of suspended lipid nanoparticles was also found by Unruh et al. (1999) for trimyristin. The decrease in melting temperature is a consequence of the crystal size. In a similar manner in the present study crystallization temperature changes from 47.2 °C in bulk to 39.8 °C in dispersion. Decreased melting temperature of suspended lipid nanoparticles was also found by Unruh & Bunjes (1999) for trimyristin. The decrease in melting temperature is a consequence of the crystal size. In a similar manner in the present study crystallization temperature changes about 7 °C from 47.2°C in bulk to 39.8°C in dispersion. Decreased crystallization temperature is a phenomenon observed in solid lipid particle dispersions which was as well attributed to small droplet size (Montenegro et al., 2003). In addition, from the melting and crystallization curves it becomes obvious that there is a polymorphic solid state present in the SSS dispersion which is not present in bulk. Based on the crystallization temperature it must be assumed that this will be the case at room temperature. Because of this polymorphism DSC results are not suitable for quantitative analysis of the SFC since usually the same average melting enthalpy is taken into account for each entity (Le Botlan et al., 1999).

The results for T_1 and T_2 measurement of the bulk material are summarized in Table 4-2 to provide the full view of NMR-relaxation in the investigated systems, which is necessary for a detailed interpretation and a dedicated data processing scheme. With 0.17 s T_1 relaxation times for bulk MCT and bulk SSS are the same, making it possible to measure with the same set of parameters of the NMR sequence. This behavior is expected, since molecules may be considered as chemically similar. T_1 of pure water (2.59 s) and the extrapolated value from ID of 2.6 s and oil/fat (~ 0.2 s) differ by one magnitude leading to the conclusion that the approach of partial signal suppression via the inversion approach is suitable for these samples.

The experimental determination of t_0 leads to a slightly smaller ID than the predicted t_0 for all samples measured (see Table 4-2). With $T_1 = 0.68$ s, T_1 of ES is significantly shorter than T_1 of water. Gum arabic type Senegal is a highly branched, long-chained polysaccharide consisting of up to ~ 90 % of arabinogalactan (Gashua et al., 2016). From the basic molecular formula of a carbohydrate ($\text{nC}_6\text{H}_{12}\text{O}_6$) it can be derived that this will make up ~ 50 % of hydrogen. Therefore, a contribution to the signal of the water phase was expected. As can be seen in Figure 4-4, t_0 decreases exponentially with increasing emulsifier content. Additionally, GA has a proteinaceous fraction of about 5 % which contributes to the decrease in T_1 as well. Lucas et al. (2005) demonstrated that protein was the factor which mostly affected the T_2 and T_1 relaxation time of the liquid water. This demonstrates the importance to carefully examine the results for determination of the emulsifier's influence.

Typically, saturation or inversion recovery is used for exact determination of T_1 . Inversion recovery for emulsions was already successfully applied by Brosio et al. (1982). The difference to the present samples is the type of emulsifier (triethanolamine stearate) and the dispersed phase volume. Brosio et al. (1982) come to the conclusion that inversion recovery is applicable for oil in water emulsions from 9 – 68 % oil content. Goudappel et al. (2001) on the other hand found that it was not possible to find a constant value for t_0 (H_2O) for mayonnaise and dressing samples, i.e. t_0 depends on the chemical composition. The signal is only partially suppressed when T_1 (H_2O) is too close to T_1 (oil). In the present study the solid and liquid oil fraction are in focus. As solid and liquid fractions show almost the same longitudinal relaxation, a partial suppression of oil signal is not substantial as it only decreases the signal-to-noise ratio (Equation (4-3)).

The screening of the dispersions for inversion delays revealed an optimum ID of 0.5 s which is slightly larger than the experimentally determined optimum zero-crossing inversion delay of 0.42 s for ES. The emulsifier concentration was shown to have a major effect on the longitudinal relaxation (Table 4-2) and therefore on t_0 . The relaxation behavior of the continuous phase of the dispersions is expected to be different from ES and water due to configurational changes of GA upon emulsification. Typically, the emulsifier is expected to migrate to the interface between water and oil due to its amphiphilic character. An effect of the emulsifier was attributed to excess emulsifier residing in the continuous phase. Therefore, ID was determined for lower concentrations than the overall concentration amounting to 10 %, 20 % and 30 % of the initial emulsifier concentration. The result indicates that the emulsifier considerably reduces the water relaxation since the optimal ID was with 0.5 s closest to the ID of 100 % ES. In theory an emulsifier diffuses to the interface and adopts the conformation that minimizes its free energy while a dynamic exchange of emulsifier molecules is possible. Biopolymers form relatively thick interfacial films with fairly open structure (McClements, 1999) allowing water molecules to migrate. Derived from the relaxation behavior molecules within these films or parts of them are still highly mobile as the water relaxation of the quasi-continuous phase is significantly reduced.

For the MCT reference emulsion 0 % solids were detected both by DSC and NMR supporting the applicability of this method (see Figure 4-6). For SSS 71.4 % solids could be found at 20°C on average. Reproducibility was measured with 20 consecutive measurements of the same sample

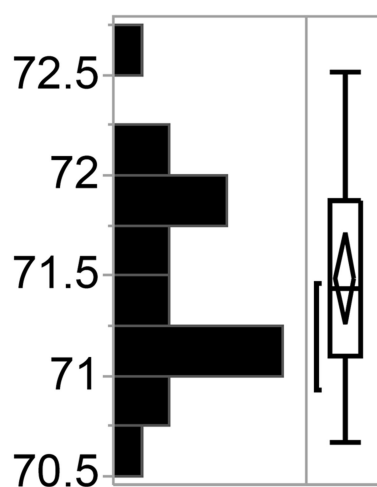


Figure 4-7: Distribution analysis of SFC [%] results for the SSS sample (number of measurements: $n = 20$).

leading to an SFC distribution. 95 % of the results are between 71.7 – 71.2 %, the standard deviation is 0.5 (see Figure 4-7). To get an idea of the SFC of the formed dispersion this is sufficient. No time or temperature influence was detected so far. . Whether the method is only highly reproducible or also accurate could be the topic of further experimental investigations. The result is in line with crystallization theory and DSC results. For crystallization to happen in every submicron emulsion droplet, a seed needs to be present in every droplet (McClements, 2012b). Development of an ordered structure reduces the molecular mobility in a way that the transverse relaxation becomes as fast as observed for a crystalline lipid. Also from the thermodynamic point of view an incomplete crystallization is expected in these highly restricted geometries. The emulsifier most likely has an effect which should be subject of further investigations. Emulsifiers are known to affect fat crystallization and fat crystal behavior in numerous ways, acting as impurities seeding nucleation and, in some cases, retarding or enhancing polymorphic transitions towards more stable forms (Coupland, 2002; Douaire et al., 2014; Cerdeira et al., 2006). From the present results the crystal structure on the microscale cannot be derived. It is not clear whether there are solid and liquid droplets or solid and liquid domains in each droplet. However, the DSC results clearly show polymorphism for SSS. Probably there is a higher molecular mobility for α -crystals than for the more stable and closer packed β -polymorph, which is partly recognized as liquid by the present approach in NMR. This hypothesis is derived from Le Botlan et al. (1999) who found the presence of a semi-solid state in milk fat, which was partially detected as liquid state by NMR. Other groups work on Transverse Relaxation Decay Deconvolution (TRDD) methods to detect polymorphism by TD-NMR (Trezza et al., 2006). So far this approach was only applied for bulk fat samples. An adaption to allow analysis of emulsions would be very helpful to get a more comprehensive picture of the internal droplet structure of dispersed fat.

4.6. Conclusion

In the present study it was demonstrated, that TD-NMR is a suitable tool for SFC determination in food emulsions and dispersions with high water content. With an inversion recovery and the proper choice of inversion delay between the 180° and 90° pulses the signal from the continuous phase could be successfully suppressed. With dedicated data processing the SFC can be

calculated. As a consequence, a quick and easy method without any sample preparation was derived. The results describe crystallization behavior of fats in dispersion in a quantitative manner which was not available before. The relaxation behavior of the emulsifier was found to be similar to the aqueous phase, which indicates that the major part of the emulsifier in the emulsion moves freely and is comparable to the signal of the emulsifier slurry without oil or fat. Future studies should determine the accuracy of the present method or expand the analytical tools to allow differentiation of the different fat polymorphs in dispersed systems as it is already demonstrated for triglycerides like SSS by TRDD.

4.7. Acknowledgments

The authors would like to thank Gerhard Krammer and the Symrise AG for supporting this work. Further, the authors would like to thank Professor John van Duynhoven from the Department of Agrotechnology and Food Science at University of Wageningen for his input and fruitful discussion. We also like to thank Jörg Müller at Bruker Biospin GmbH, Rheinstetten, for technical support.

4.8. Conflict of interest

The authors declare that they have no conflict of interest.

5. Manuscript IV: Re-designing clouds to increase turbidity in beverage emulsions

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The final publication is available at Springer via <https://doi.org/10.1007/s11483-018-9515-x>

Food Biophysics (2018) 13:91

DOI: 10.1007/s11483-018-9515-x.

5.1. Abstract

Clouds represent oil-in-water emulsions, which are used to create a turbid appearance in beverage emulsions. The aim of the present study was to investigate whether crystalline structures at the oil-water interface or a solidified dispersed phase sufficiently increase the refractive index difference between the two phases to create turbidity in a more efficient manner. Bulk materials and dispersions were characterized by differential scanning calorimetry and time-domain-NMR for crystal structure and solid fat content, respectively. Use of high melting emulsifiers (HME), 1% sodium stearoyl lactylate (SSL), 1% glyceryl stearoyl citrate (GSC), respectively 1.5% phospholipid (PL) did not result in an increase in turbidity. Data indicate that the emulsifiers did not crystallize at the interface. However, modification of the dispersed phase with high melting lipids (HML) was successful. Using fats with increasing melting point, it was shown that turbidity increased with increasing degree of crystallinity. Compared to a liquid medium chain triglyceride (MCT) turbidity increased significantly from 290 NTU (ratio) using hydrogenated palm fat (HPF, 400 NTU (ratio)) or tristearin (SSS, 440 NTU (ratio)) instead. In order to induce that effect, a critical degree of crystallinity of more than 36% needed to be achieved.

Keywords: Cloud; turbidity; solid lipid particles; crystallization; beverage emulsion; time-domain NMR

5.2. Acknowledgment

The authors would like to thank Gerhard Krammer and the Symrise AG for supporting this work. Further, the authors would like to thank Professor Gisela Guthausen from Institute of Mechanical Process Engineering and Mechanics (MVM), Karlsruher Institut für Technologie (KIT), for her input and fruitful discussion on TD-NMR results.

5.3. Introduction

Consumer's expectation of a fruit-based beverage is a turbid appearance. Therefore, the visual appearance of a cloudy drink is a critical factor for consumer acceptance (Shachman, 2004). Turbidity may be achieved by the addition of a so-called cloud. A cloud is a concentrated oil-in-

water (o/w) emulsion which will be diluted in the final beverage application (Tan, 1998; Buffo et al., 2001). It typically consists of a preserved water phase, a food-grade emulsifier such as gum arabic (GA) or modified starch and a dispersed oil phase consisting of citrus oil or medium chain triglycerides (Shachman, 2004b; Buffo et al., 2002). Typically, weighting agents like wood resins are used to increase the density of the dispersed phase to enhance the performance of a beverage emulsion (Given, 2009).

Turbidity is caused by light scattering at the oil droplet interface (Hernandez & Baker, 1991; Hernandez et al., 1991). Previous studies on beverage emulsions focused on the influence of the oil droplet size and concentration on turbidity (Mirhosseini et al., 2008b; Taherian et al., 2006). It became obvious that a change in droplet size may be used to optimize scattering efficiency. In a systematic approach we found that maximum scattering efficiency is achieved at a mean surface-weighted droplet size of 0.2 μm (Linke & Drusch, 2016). Taking into account that beverage emulsions are typically produced with a maximum droplet size below 1 μm , results from the study show that an increase in turbidity of 20 % may be achieved by optimization of the oil droplet size. The correlation between oil concentration and turbidity is linear and therefore intensive work was done to maximize oil load by careful choice and optimization of emulsifiers (Buffo & Reineccius, 2001; Almeida et al., 2015; Gharibzahedi et al., 2012; Jacome-Guth et al., 1991). However, at high oil content physical stability becomes an issue and sensory properties may be negatively affected. When investigating scattering efficiency in cloudy apple juice, Benitez et al. (Benitez et al., 2007) reported that the refractive index difference (RID) between serum phase and cloud particles affects turbidity. Our recent study highlights the importance of the RID to be the driving factor for turbidity in beverage emulsions (Linke & Drusch, 2016). The RID between an oil phase with wood rosin as weighting agent and the corresponding non-weighted oil phase amounted to 0.04 and led to an increase in turbidity by 72 %. Therefore, increasing the refractive index (RI) of the dispersed phase seems to be a promising strategy to increase the turbidity of a cloud. However, presence of a weighting agent and other food additives contradicts the consumer demand for clean label foods. It is therefore a major challenge to deliver products that perform on the same level or better to be able to replace these additives.

It is generally known that the RI of a material increases when the state changes from liquid to solid. The solid i.e. crystalline form of a substance is the state of highest order. Since the phase relations in a crystal lattice are fixed, this leads to stronger external and internal interference of

the light with the material compared to a liquid (Giacovazzo, 2005). This physical phenomenon was e.g. observed by Helgason et al. (2015), who reported that spectrophotometric absorbance readings increased upon crystallization of dispersed fat droplets in octadecane nanoemulsions. Therefore, the aim of the present study was to confirm our hypothesis that turbidity of a cloud may be increased by changing the RID either by using an emulsifier in the solid state at the interface or via a solidified dispersed phase. Dispersions were prepared via the melt-emulsification method described by Siekmann & Westesen, (1994). Particle size distribution, turbidity and crystal structure of the lipids were analyzed to characterize the dispersions. TD-NMR was used to assess whether the solid fat content (SFC) in the dispersion correlates with turbidity of the system. Since crystallization greatly depends on temperature, the influence of the storage temperature was evaluated additionally.

5.4. Material and methods

All materials were either purchased or kindly provided by the suppliers mentioned below: Gum Arabic Senegal type (GA, Nexira GmbH, München, Germany), octenyl-succinate derivatized starch refined from waxy maize (OSA) and Quillaja extract (Q, Ingredion Germany GmbH, Hamburg, Germany), sodium stearoyl lactylate Grindsted R 55 (SSL, Danisco A/S, Copenhagen, Denmark), phospholipid Phospholipon 80H (PL, Lipoid GmbH, Ludwigshafen, Germany), potassium sorbate (Eastman Chemical Company, Kingsport, USA) and citric acid (Jungbunzlauer Suisse AG, Basel, Switzerland), glyceryl stearoyl citrate Imwitor 372P (GSC), SSS, hydrogenated palm fat (HPF), hydrogenated palm kernel fat (HPK) a mixture of palm and palm kernel fat (PPK) and medium chain triglyceride (MCT), (Cremer Oleo GmbH & Co. KG, Hamburg, Germany).

5.4.1. Characterization of the bulk materials

The fatty acid composition of the lipids was determined by gas chromatographic analysis of the fatty acid methyl esters according to DIN EN 14103 (2015). Chromatography was conducted using an Agilent hp6890 with a 20 m DB-23 column (both Agilent Technologies, Santa Clara,

USA). HMLs were molten prior to analysis. A cold injection system was used. Allocation was done via retention indices.

The RI of the solid and molten lipids were determined by Schmidt & Haensch (Berlin, Germany) using an ATR-B refractometer. For the measurement, the solid lipid was applied to the prism until it was completely covered. The prism was heated up until the lipid was fully molten and the refractive index was measured at a wavelength of 589 nm. Thereafter the prism was cooled down to 20 °C and the refractive index of the solid lipid was determined. 10 recordings per sample were made and the average and standard deviation were calculated. Analysis of the refractive index for the phospholipid in the solid or molten state was not possible, since it was not completely molten at maximum operation temperature of the refractometer (100 °C) and did not crystallize homogenously according to Schmidt & Haensch..

The SFC was evaluated with a 20 MHz low resolution NMR “Bruker the minispec” mq20 with a H20-10-25(33)RVGX probe (Bruker BioSpin GmbH, Rheinstetten, Germany). Data were acquired using “*the minispec Software*” V2.59Rev06/NT. The raw material was analyzed according to the indirect method (DIN EN ISO 8292-2:2008:, 2010b) since the melting temperature of HPF and SSS were out of range for the direct method. Analysis of the molten state was done at 90 °C, the SFC was determined at 20 °C. The manufacturers certificated standard set E1201050 was used to calibrate the instrument.

To determine the melting and crystallization behavior, bulk materials were further characterized with a 200 F3 Maia, Netzsch calorimeter using low pressure closed lid aluminium pans (both Erich Netzsch GmbH & Co. Holding KG, Selb, Germany). Melting point and the enthalpy of melting of the raw material were determined with 8 – 10 mg of sample. The samples were heated from 20 °C or 4 °C, respectively, to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min.

5.4.2. Preparation of emulsions and dispersions

A reference emulsion representing a typical cloud was prepared by dissolving gum arabic (13.5 %w/w) and preservatives in water with a RW20DZM agitator equipped with a R 1302 dissolver stirrer (IKA®-Werke GmbH & Co. KG, Staufen, Germany). The aqueous solution was

left over night for hydration. MCT oil (7.15 % w/w) was gradually added to the continuous phase while mixing the solution with an Ultra-Turrax T50 with dispersion unit S50N (IKA®-Werke GmbH & Co. KG, Staufen, Germany) at 6000 rpm for 5 minutes to generate a coarse emulsion. The coarse emulsion was left at least for two hours for de-aeration before homogenization with a laboratory table top model homogenizer NS1001L2K (GEA Niro Soavi S.p.A., Parma, Italy). Homogenization was done with three cycles in a two stage process at 50/5 MPa.

For each individual formulation the first and second pressure stage were adjusted to achieve a mono-modal droplet size distribution with a surface weighted mean diameter of $\bar{x}_{1,2} = 0.2 \mu\text{m}$. Previous studies showed that this is the size range with maximum scattering efficiency (Linke & Drusch, 2016). More specifically, for HME aqueous surfactant solutions were prepared by mixing the specified amount of emulsifier (Table 5-1) in deionized water. Emulsifier concentration was chosen according to manufacturers' recommendation. Temperature was adjusted to 10 °C above the melting temperature of the emulsifier and the solution was stirred at 2000 rpm until the emulsifier was fully dispersed. MCT was heated to the same temperature as

Table 5-1: Formulations of prepared dispersions with different emulsifier [phospholipon (PL), sodium stearyl lactylate (SSL), glyceryl stearyl citrate (GSC)] or different dispersed phase [medium chain triglyceride (MCT), hydrogenated palm kernel fat (HPK), palm & palm kernel fat (PPK), hydrogenated palm fat (HPF), tristearin (SSS)]

Sample	Emulsifying agent [w/w%]	Lipid [v/w%]	Type of emulsifier	Type of lipid
Reference	13.5	7.15	Gum arabic	MCT
HPK	13.5	6.8	Gum arabic	HPK
PPK	13.5	6.7	Gum arabic	PPK
HPF	13.5	6.7	Gum arabic	HPF
SSS	13.5	6.6	Gum arabic	SSS
SSL	1.0	7.15	SSL	MCT
GSC	1.0	7.15	GSC	MCT
PL	1.5	7.15	PL	MCT

surfactant solution. After pre-homogenization the emulsion was kept for 30 min 10 °C above melting temperature under constant gentle stirring (200 rpm, magnetic stir bar). Pre-homogenization and homogenization was performed as outlined above.

The samples containing other lipids than MCT oil as dispersed phase were prepared via a hot high pressure homogenization method as proposed by (Siekman & Westesen, 1994). These samples do not belong to classical emulsions and are therefore named dispersions in the following. For HMLs the aqueous phase was heated to 10 °C above the melting point of the used lipid and stirred. The lipid phase was fully molten in a water bath at 90 °C. The amount of dispersed phase was adjusted volumetrically after volumetric determination of the density of the molten lipids (data not shown). After pre-homogenization the dispersion was kept for 30 min 10 °C above melting temperature under constant gentle stirring (200 rpm, magnetic stir bar). Pre-homogenization and homogenization was performed as outlined above. To prevent premature crystallization the homogenizer was preheated with 90 °C hot water.

After homogenization the dispersion was filled in two bottles, one was stored at room temperature the other was stored at 4 °C in the refrigerator. All dispersions were left 40 h to allow solidification of the lipid phase before further analysis. This procedure is adopted from SFC measurement (DIN EN ISO 8292-2:2008:, 2010b). Samples were analyzed for droplet size, crystallization behavior and turbidity after 7 d, 14 d, 28 d and 56 d. All dispersions were prepared in duplicate. Formulations are shown in Table 5-1.

5.4.3. Physical characterization of the dispersions

Droplet size distribution was analyzed via laser diffraction using a Malvern Mastersizer 3000 equipped with a dispersion unit Hydro MV (both Malvern Instruments Ltd, Worcestershire, UK). Laser diffraction measurement was undertaken and reported following (ISO 13320:2009, 2009) and (ISO 9276-2:2014-05, 2014). For each sample five measurements were made using the following parameters: pump speed was set at 2000 rpm, measurement delay was 2 seconds, measurement time was 10 seconds and 10,000 snaps were taken. For each emulsion and dispersion the optical parameters were adjusted according to the RI as shown in Table 5-2 maintaining the values for residual and weighted residual as low as possible. The optical

parameters of the phospholipid dispersion were established using the optical properties optimizer of the Mastersizer 3000 using software version 3.3. Droplet size was additionally measured immediately after dispersion preparation to ensure that the surface-weighted mean diameter was close to 0.2 μm . Melting and crystallization behavior of the emulsifier and lipid phase in the dispersion was analyzed via DSC with 22 – 27 mg sample as outlined above.

Table 5-2: Refractive index (RI) in solid and molten state, solid fat content (SFC) (indirect method), peak melting (T_m) and crystallization temperatures (T_c) of high-melting emulsifiers (sodium stearyl lactylate (SSL), glyceryl stearyl citrate (GSC), phospholipon (PL)) and different lipids (medium chain triglyceride (MCT), hydrogenated palm kernel fat (HPK), palm & palm kernel fat (PPK), hydrogenated palm fat (HPF), tristearin (SSS))

Sample	RI _{solid} *	RI _{molten} *	SFC _{indirect} [%]	T _{mPeak1} [°C]	T _{mPeak2} [°C]	T _c Peak1 [°C]	T _c Peak2 [°C]
SSL	1.51 ± 0.00	1.45 ± 0.00	-	49.5 49.9	70.3 69.9	41.7 41.8	-
GSC	1.52 ± 0.00	1.45 ± 0.00	-	62.7 62.1	-	52.8 52.7	49.2 49.2
PL	1.50	n.d.	-	83.1 83.6	-	77.2 77.3	-
MCT	-	1.45 ± 0.00	0.09 0.07	< 0	-	< 0	-
HPK	1.50 ± 0.00	1.45 ± 0.00	96.25 96.24	39.4 38.7	-	17.6 17.7	14.4 14.4
PPK	1.51 ± 0.00	1.45 ± 0.00	97.38 97.25	45.5 45.5	-	28.7 28.8	19.6 19.7
HPF	1.51 ± 0.00	1.44 ± 0.00	98.47 98.44	49.7 49.6	60.8 61.3	41.8 41.9	-
SSS	1.52 ± 0.00	1.44 ± 0.00	98.85 98.83	74.7 74.5	-	47.1 47.2	-

* ± SD for n = 10

The SFC of the HML dispersions was evaluated with a 20 MHz low resolution NMR “Bruker the minispec” mq20 with a H20-10-25(33)RVGX probe (Bruker BioSpin GmbH, Rheinstetten, Germany). The dispersions were analyzed using an inversion recovery experiment (Linke, Guthausen, Flöter & Drusch, accepted manuscript ejlt.201700132.R3). The inversion delay was determined for each dispersed phase (SSS: 530 ms; HPF: 560 ms; PPK: 560 ms; HPK: 520 ms). Acquisition and timing parameters were optimized using the aqueous phase with dissolved preservatives as reference sample, since most of the signal will result from this phase. The probe was tempered to 20 °C with a cryostat CP25 (Thermo Haake GmbH, Karlsruhe, Germany). Air was flushed through the probe to prevent condensation. A 1 cm spacer (Bruker) was inserted into the probe to position the sample tube in the homogeneous region of the radio frequency (RF) coil. 1 cm (350 µl) of sample was pipetted into glass inserts with bottom which are placed in 10 mm test tubes. Inserts are sealed with parafilm, test tubes are closed with caps (tubes, inserts and caps purchased from Bruker). All data analysis was executed in *JMP® 13.0.0*, (SAS Institute Inc., Cary, USA). For each dispersion and storage temperature three test tubes were prepared after homogenization.

For turbidity measurement emulsions were diluted to a typical application concentration of 0.2 % in filtered water and left to settle for approx. 30 minutes prior to turbidity measurement. Turbidity was measured using a 2100AN IS laboratory turbidimeter (DIN EN ISO 7027:2000-04) compatible with infrared light source at 870 nm ± 30 LED (Hach Lange GmbH, Berlin, Germany) with four detectors measuring 90°, 180°, forward and backward scattering. Readings are recorded in NTU (ratio) – mode, which is a ratio of the signals of all four detectors. All measurements were done in triplicate after 40 h, 7 d, 14 d, 28 d and 56 d.

5.4.4. Statistical analysis

All measurements were at least done in duplicate. All means and standard deviations were calculated in Microsoft®Excel (Microsoft Office 2010).

5.5. Results and Discussion

As outlined in the introduction the aim of the present work was to show whether an emulsifier in the solid state at the interface or a solid dispersed phase increases the turbidity of a beverage

cloud. Results of the experiments on HMEs are described first followed by the results on HMLs as solid dispersed phase.

Prior to preparation of the emulsions bulk properties of the HMEs were analyzed. HMEs comprised SSL, PL and GSC. Phase transition behavior was analyzed by DSC (Figure 5-1). Results revealed two melting events for SSL with peak temperatures of 49.5 °C and 70.3 °C, respectively. Crystallization peak temperature was 41.7 °C. For GSC the peak melting and crystallization temperatures were 62.7 °C and 52.8 °C, respectively. PL showed a wide melting range (30-95 °C) with a maximum at 83.1 °C. It needs to be kept in mind that the melting point of phospholipids may be interpreted with caution because the melting behavior is superseded by a chemical degradation process (Bueschelberger, 2008). The peak crystallization temperature of PL is 77.2 °C. These data clearly show that crystallization occurs well above room temperature, which is a pre-requisite for solidification at the interface. Analysis of the RI determination is presented in Table 5-2. With 1.50 – 1.52 the RI in the solid state is well above the RI of the oil phase (1.45). Based on the results of Linke & Drusch (2016) it can be concluded that an increase in the RID of 0.05 – 0.07 significantly increases turbidity in beverage emulsions.

When preparing emulsions, homogenization conditions were adjusted to achieve an oil droplet size with maximum scattering efficiency. The mean surface weighted droplet size of the dispersions was in a similar range between 0.1 – 0.2 µm (Table 5-3). Therefore the dispersions

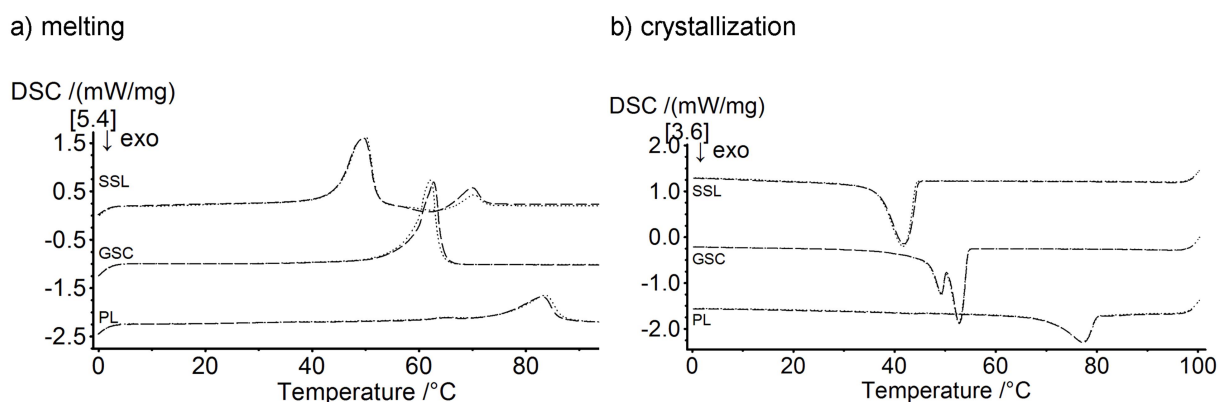


Figure 5-1: DSC (a) melting and (b) crystallization thermogram of bulk sodium stearyl lactylate (SSL), glyceryl stearyl citrate (GSC) and phospholipid (PL). The samples were heated from 0 °C to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min.

Table 5-3: Droplet size ($\bar{x}_{1,2}$), turbidity [NTU (ratio)], peak melting (T_m) and crystallization temperatures (T_c) and SFC of dispersions prepared with gum arabic and medium chain triglyceride (MCT), hydrogenated palm kernel fat (HPK), palm & palm kernel fat (PPK), hydrogenated palm fat (HPF) and tristearin (SSS); or sodium stearyl lactylate (SSL), glyceryl stearyl citrate (GSC), phospholipon (PL) and MCT

Sample	$\bar{x}_{1,2}$ * [μm]	Turbidity** [NTU (ratio)]	$T_{m\text{Peak}1}$ [$^{\circ}\text{C}$]	$T_{m\text{Peak}2}$ [$^{\circ}\text{C}$]	T_c Peak1 [$^{\circ}\text{C}$]	T_c Peak2 [$^{\circ}\text{C}$]	SFC** [%]
SSL	0.2 ± 0.03	210 ± 2.1	-	-	-	-	-
GSC	0.2 ± 0.02	218 ± 6.2	-	-	-	-	-
PL	0.1 ± 0.08	121 ± 6.0	-	-	-	-	-
MCT	0.3 ± 0.01	292 ± 1.5	< 0	-	< 0	-	-
HPK	0.2 ± 0.00	276 ± 3.8	32.1 31.8	-	9.2 9.7	-	28 ± 4.40
PPK	0.2 ± 0.00	293 ± 3.8	39.2 38.8	-	27.8 27.1	19.3 15.0	36 ± 2.74
HPF	0.2 ± 0.04	398 ± 23.6	48.4 47.8	58.3 58.9	34.9 -	42.2 41.4	54 ± 2.78
SSS	0.1 ± 0.00	441 ± 6.3	54.2 57.4	70.6 70.4	40.1 40.2	-	65 ± 3.63

* $\pm$ SD for n = 5; ** $\pm$ SD for n = 6

are in the size range, which induces maximum scattering effects and the droplet size should not be a major influencing factor in the present study. Emulsions with PL as emulsifier showed a phase separation immediately after preparation. These results are in line with other published data reporting similar problems when stabilizing so-called solid lipid nanoparticles (SLN) with phospholipids (Salminen et al., 2013; Salminen et al., 2014b; Salminen et al., 2014a; Westesen et al., 1997). Upon homogenization oil droplets become smaller and the droplet interfacial area increases. In bulk, Phospholipids form bilayers and lamellar vesicles which reduces their mobility (Garti & Yano, 2001). Therefore, phospholipids are too slow to cover the newly formed interface immediately after homogenization (Westesen & Siekmann, 1997). Amphiphilic co-surfactants can help to disrupt the vesicles and rapidly adsorb on the uncovered interface of the oil droplets (Salminen et al., 2014a). Therefore, as food-grade approach, Quillaja extract (1.5 w/w%) was

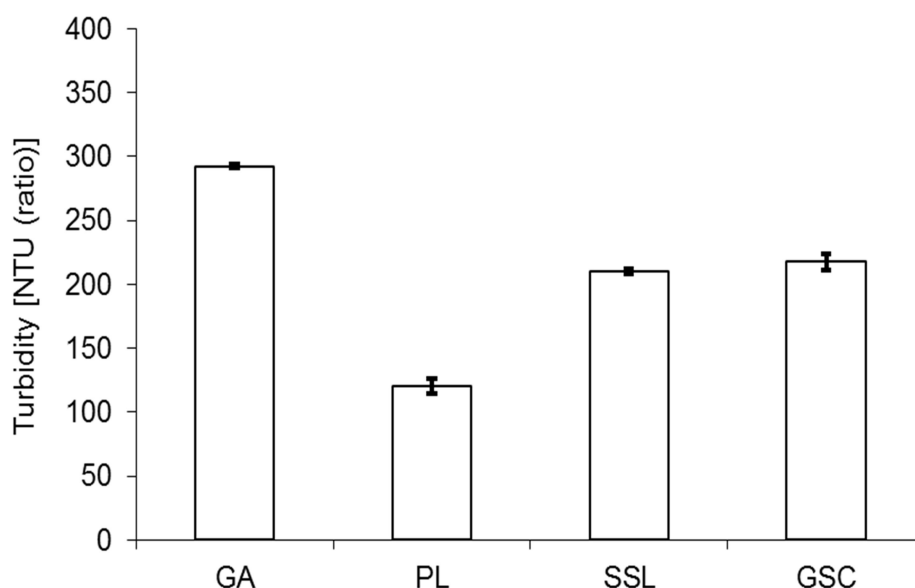


Figure 5-2: Turbidity [NTU (ratio)] of 0.2 % concentrations of dispersions with different high melting emulsifiers (GA = gum arabic; PL = phospholipid; SSL = sodium stearyl lactylate; GSC = glyceryl stearyl citrate). All data are means $\pm$ standard deviation for $n = 6$.

used as co-surfactant for additional stabilization.

The turbidity of diluted emulsions with different high-melting emulsifiers is shown in Table 5-3 and Figure 5-2. It becomes obvious that all emulsions are less turbid than the reference emulsion based on GA. The emulsion with PL as emulsifier is the least turbid with 121 NTU (ratio) which is less than half compared to the reference with 292 NTU (ratio). Emulsions prepared with SSL and GSC show similar turbidity with 210 and 218 NTU (ratio), respectively. From previous work we know that a difference of 50 NTU (ratio) is perceived as significantly different by the human eye in this turbidity range (Linke & Drusch, 2016). Thus, the reference emulsion will be perceived as more turbid than emulsions based on HMEs.

At first sight, the results contradict the initial hypothesis of an increased light scattering when a solidified emulsifier with high RI is present at the interface. However, DSC thermograms of the emulsions do not show any melting or crystallization events (Figure 5-3). This might either be due to a lack of sensitivity of the DSC (surfactant concentration is very low with 1 – 1.5 %) or must be interpreted in a way that crystallization of the used emulsifier does not occur. A test with 1 % PPK in water clearly showed a detectable peak in the thermogram, which confirms that instrument sensitivity is sufficient to detect complete crystallization the emulsifier (data not

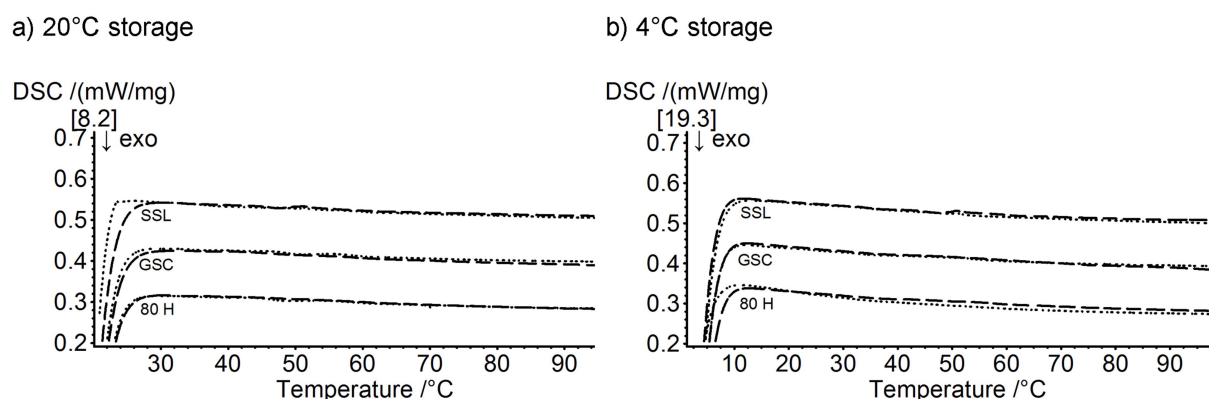


Figure 5-3: DSC melting thermogram of emulsions stabilized with sodium stearyl lactylate (SSL), glyceryl stearyl citrate (GSC) and phospholipid (PL) stored at (a) 20 °C and (b) 4 °C. Samples were heated from 20 °C or 4 °C, respectively, to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min

shown). Therefore, it must be hypothesized that the emulsifier did not or only partly crystallize at the interface. Kurukji et al. (2013) detected a melting peak in SSL stabilized sunflower-oil in water emulsions at similar concentration. However, they prepared SSL aggregates which were used as Pickering particles and stored emulsions at 3 °C. Additionally, they used a different DSC with sample volumes of 700 mg. In the present study, samples stored at 4 °C did not show melting events for any emulsifier (Figure 5-3b). Therefore, it must be concluded that the emulsifier either does only solidify to an extent that cannot be detected by DSC or conformational changes at the interface inhibit the formation of a crystal network if not aggregated before emulsification. Considering that the emulsifier initially forms a monolayer at the interface and needs to adapt to the curvature of the droplet interface, interactions of the molecules with each other may significantly differ to their bulk state. For PL molecular arrangement is also affected by the presence of the co-surfactant, which might explain the low turbidity of this sample.

Assuming that the emulsifier at the interface did not affect turbidity, it remains to be discussed, why GA-based emulsions showed a higher turbidity than all other emulsions. All samples contained the same type and amount of oil and oil droplet size was in a similar range. GA is the only emulsifier with a high molecular weight in this set of emulsifiers and was used in a significantly higher concentration to stabilize the emulsion. In the case of high molecular weight emulsifiers it is known that an excess contributes to turbidity (Linke & Drusch, 2016) and thus in the present study GA in the bulk contributed to the scattering efficiency of the system. In

summary the hypothesis on the impact of high-melting emulsifiers on turbidity was not verified in the present study, most likely due to insufficient crystallization of the emulsifier at the interface. Since increasing emulsifier content and thus excess use of an additive may bear legal problems and is not in line with good manufacturing practice, in terms of the aim to redesign clouds, use of high-melting emulsifiers is no suitable strategy.

In an alternative approach MCT was replaced by lipids with differing melting point as dispersed phase in the cloud. These lipids comprised tristearin (SSS), hydrogenated palm fat (HPF), hydrogenated palm kernel fat (HPK) and a mixture of palm and palm kernel fat (PPK). The characterization of the bulk materials is provided in Table 5-2. RID ranged from 0.05 to 0.08 and the RI in the solid state is higher than the RI of MCT, which remains in the liquid state at room temperature. The melting temperature increased from HPK < PPK < HPF < SSS. All values are well above room temperature and SFC determination of the bulk raw material at room temperature was above 96 % for all four fats and below 0.1 % for MCT (Table 5-2). FAME analysis confirmed that HPK mainly consisted of saturated fatty acids containing 1 % of capric acid (C10), 55 % lauric acid (C12), 20 % myristic acid (C14), 10 % palmitic acid (C16) and 12 % stearic acid (C18). PPK exhibited a similar fatty acid spectrum with differences in quantitative composition: 35 % of C12, 14 % C14, 19 % C16 and 27 % C18. HPF represented a mixture of triglycerides with two high melting fatty acids: 45 % C16 and 55 % C18. SSS represents a mono-acid triglyceride consisting only of C18.

Surface weighted mean diameter of the dispersions with high melting lipids and MCT-emulsions ranged from 0.1 – 0.3 μm (Table 5-3). Turbidity heavily depended on the type of dispersed phase as shown in Table 5-3 and Figure 5-4. The reference emulsion with MCT exhibited a turbidity of 292 NTU (ratio). Dispersions prepared with HPF and SSS were significantly more turbid than the reference with 398 NTU (ratio) and 441 NTU (ratio). Nevertheless no direct correlation with the melting point of the raw material could be established since dispersions with HPK and PPK were close to the reference emulsion with 276 NTU (ratio) and 293 NTU (ratio), respectively. DSC melting thermograms (Figure 5-5a) of the dispersions with HMF show melting events for all samples but the liquid reference (MCT). Looking at the crystallization thermograms (Figure 5-5b) becomes obvious that HPF and SSS start to crystallize above 40 °C while crystallization of PPK starts below 30 °C and HPK below 20 °C. Even though HPK peak

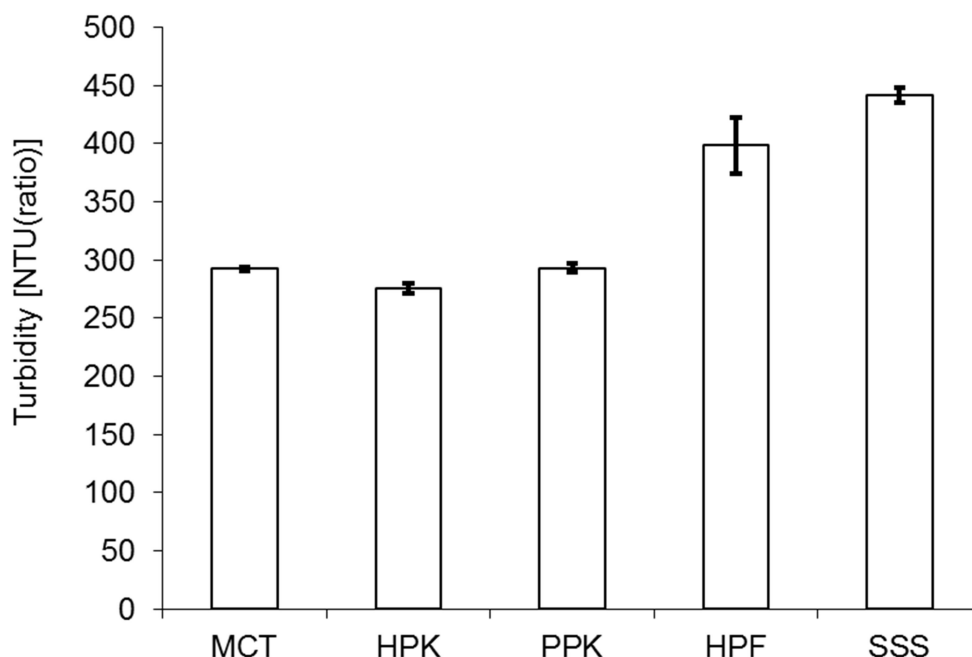


Figure 5-4: Turbidity [NTU (ratio)] of 0.2% concentrations of reference MCT emulsion and dispersions with different high melting lipids (MCT = medium chain triglyceride; HPK = hydrogenated palm kernel fat; PPK = mixture of palm & palm kernel fat; HPF = hydrogenated palm fat; SSS = tristearin. All data are means $\pm$ standard deviation for $n = 6$

crystallization temperature is below 10 °C isothermal crystallization at room temperature for 40 h led to at least partial crystallization.

For the development of the SFC after hot emulsification the crystallization temperature is a more appropriate bulk characteristic. As outlined above for HPK and PPK, crystallization events occur close to room temperature and therefore the dispersed phase most likely was only partly in the crystalline state. Nevertheless, results indicate that if crystalline materials dominate the dispersed phase, turbidity can be increased in a beverage emulsion. Therefore we decided to investigate the physical state of the dispersed phase in more detail. To get a first indication if solidification of the dispersed phase is responsible for the increase in turbidity, turbidity of a SSS-dispersion was measured while varying the temperature. The cuvette for turbidity measurement was tempered in a water bath for one hour prior to measurement. Starting from 20 °C temperature was increased in decades up to 80 °C. The results can be found in the supplementary material, Figure 5-8). In this experiment turbidity slightly decreased until 50 °C were reached. At 60 °C turbidity

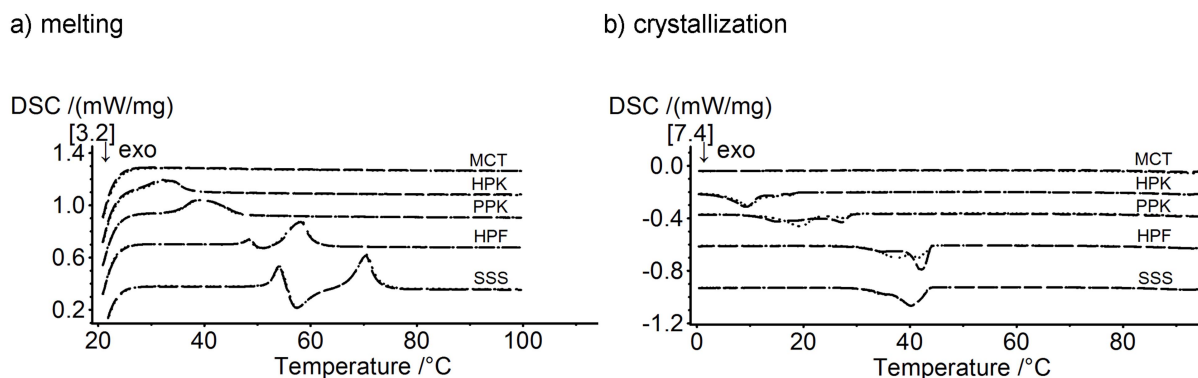


Figure 5-5: DSC (a) melting and (b) crystallization thermograms of reference emulsion with medium chain triglyceride (MCT) and dispersion with hydrogenated palm kernel fat (HPK), mixture of palm & palm kernel fat (PPK), hydrogenated palm fat (HPF) and tristearin (SSS). Samples were heated from 20 °C or 4 °C, respectively, to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min

increased back to its initial value and a finally dropped to approximately 240 NTU (ratio) at 70 °C and 80 °C. For isothermal treatment of SSS the α -polymorph was found to melt at 54.9 °C and the β -polymorph was found to crystallize at 61 °C in bulk (Da Silva et al., 2009) which correlates well with the measured T_{mPeak1} (Table 5-3) and the recorded DSC thermogram for the SSS sample (Figure 5-5a). As can be seen in Figure 5-5a melting of the SSS sample starts above 50 °C which explains the low degree of loss in turbidity up to 50 °C. The increase in turbidity at 60 °C might arise from α to β transition. The measured T_{mPeak2} (Table 5-3) of 71 °C correlates well with the loss in turbidity. In summary, change of turbidity with temperature supports the hypothesis that an increase in turbidity can be achieved by a solidifying the dispersed phase.

Before discussing the impact of the SFC on turbidity it is necessary to provide some background on crystallization in dispersed systems. It is known that crystallization in dispersion differs remarkably from crystallization in bulk (Westesen et al., 1997; Garti & Yano, 2001; Povey, 2001; McClements et al., 1993). The very general relationship between droplet size reduction of the dispersed phase and decrease in droplet volume, increase in surface to volume ratio and increase in droplet number has recently been summed up by (McClements, 2012b) making it very obvious that the physical properties will differ from bulk. Melting of nano-ranged lipids is size dependent (Unruh et al., 1999). It was shown that the melting temperature of suspensions of trimyristin nanoparticles decreases with decreasing particle thickness. In the present study was observed as

well a decrease in melting temperatures in dispersion compared to bulk (Table 5-2, Table 5-3). Solidification of lipids is induced either by homogeneous or heterogeneous crystallization (Aquilano & Sgualdino, 2001). While homogeneous nucleation occurs in a supersaturated melt when no impurities are present, heterogeneous nucleation is induced by impurities or surfaces (McClements, 2012b). The creation of a large number of small droplets inevitable leads to varying amounts of these catalysts in each single droplet. This typically induces a greatly increased degree of supercooling of up to 20 °C and rather homogeneous nucleation (Montenegro et al., 2003). These considerations have not yet accounted for the effect the emulsifier might imply to the system. Depending on the structure and chemical similarity to the lipid the emulsifier can act as template and therefore accelerate heterogeneous crystallization (Katsuragi et al., 2001; Kalnin et al., 2004; Sakamoto et al., 2004). On the other hand the emulsifier was found to inhibit crystal growth (Garti & Yano, 2001; Bunjes & Koch, 2005). It becomes obvious that lipid crystallization and in particular crystallization in dispersed systems is a multivariable and complex process.

These considerations lead to the conclusion that the SFC of the dispersions cannot be predicted from bulk properties, but rather needs to be characterized *in situ* to be able to prove the hypothesis. A first quantitative proof of the SFC is the existence of thermal events in the DSC as represented by endothermal melting events during DSC analysis (Table 5-3). SFC determination in bulk fats and oils with low resolution or time-domain nuclear magnetic resonance (TD-NMR) is well established and standard procedures are available (DIN EN ISO 8292-1:2010-08, 2010a; DIN EN ISO 8292-2:2008, 2010a; International Union of Pure and Applied Chemistry, 1992; The American Oil Chemists' Society, Reapproved 2009a, Reapproved 2009b). Low water foods with water content up to 10–15% can be analyzed by conventional TD-NMR methods based on transverse relaxation differences (Todt et al., 2006). However, for SFC determination in food emulsions or SLNs, problems arise insulating the dispersed (droplet) phase signal from the quasi-continuous phase (Johns, 2009). In addition, the transverse water relaxation is strongly affected by the food network structure (Gianferri et al., 2007), which complicates a direct SFC-like measurement combined with free induction decay (FID) deconvolution. Therefore, to quantify the SFC in dispersions with a high water content and a specific method was developed (Linke, Guthausen, Flöter & Drusch, accepted manuscript ejlt.201700132.R3). Adopted from classical SFC measurement the signal from the continuous phase is removed by a preset 180° pulse which

inverts the magnetization. With a properly chosen inversion delay protons of the continuous phase pass through zero magnetization at the time of the subsequent 90° pulse. At this stage only the recovered dispersed phase will be excited and the resulting free induction decay can be analyzed for its solid and liquid contributions. In the present study The SFC was measured after 40 h and the results are displayed in Table 5-3 and Figure 5-6. With increasing melting point of the dispersed phase the SFC increases in the dispersion. HPK and PPK show a low SFC with 28 % and 36 %, respectively. In contrast, HPF and SSS exhibit a markedly higher SFC of 54 % and 65 %, respectively. The low SFC in HPK and PPK dispersion is due to their lower crystallization temperatures. While for HPF and SSS peak crystallization temperature is above 40 °C and is terminated before 30 °C, HPK and PPK crystallize around and below room temperature (Fig. 5b). Thus, the storage temperature is too high for HPK and PPK to fully crystallize. For HPF and SSS incomplete crystallization cannot be caused by temperature but must be due to different crystallization of the dispersed state compared to bulk. It remains unclear though if only droplets with a seed crystal crystallized or if all droplets are partially crystalline. Since only samples with a SFC above 50 % are significantly more turbid than the liquid reference,

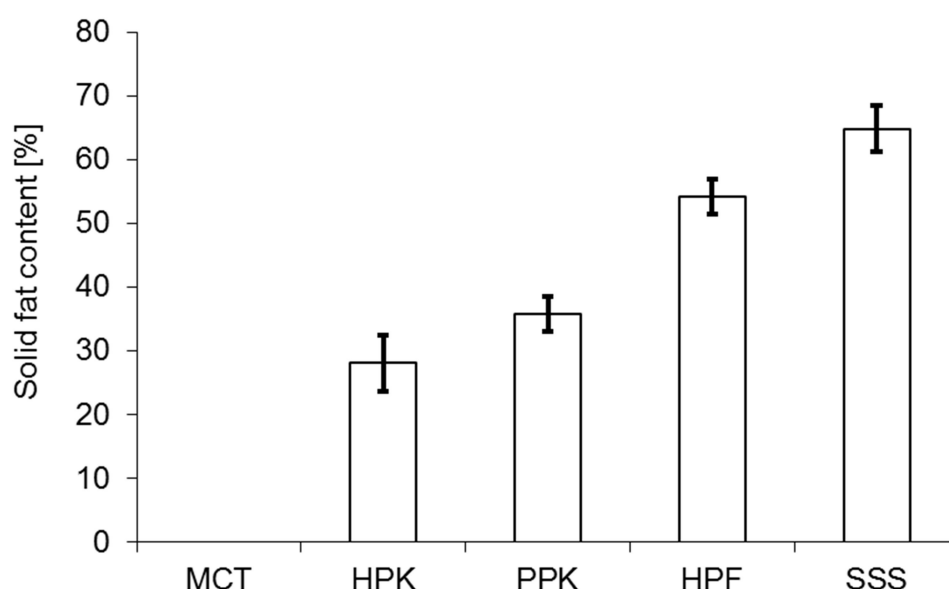


Figure 5-6: Solid fat content (SFC) of dispersions with different high melting lipids (MCT = medium chain triglyceride; HPK = hydrogenated palm kernel fat; PPK = mixture of palm & palm kernel fat; HPF = hydrogenated palm fat; SSS = tristearin. All data are means $\pm$ standard deviation for $n = 6$.

results suggests that the turbidity enhancing effect evolves only above a critical minimum crystallinity within the system.

A final aspect is the physical stability of the dispersion. Clouds need to be stable in the final beverage for at least six month. Since the SSS-based dispersion exhibited improved turbidity and had better reproducibility than HPF the stability and crystal structure of those samples were observed over a prolonged time period either stored at room temperature or at 4 °C. Samples stored at room temperature were stable for eight weeks without significant change in droplet size and only a minor change in turbidity after four months (Table 5-4). After two month the dispersion had changed into a gel-like state. This behavior was also observed by (Freitas & Müller, 1999; Helgason et al., 2009; Seetapan et al., 2010; Westesen et al., 1997; Westesen & Siekmann, 1997; Fuller et al., 2015). As a consequence of the gelformation, the cloud is not

Table 5-4: Particle size ($\bar{x}_{1,2}$), turbidity and percental melting enthalpy of α - and β -polymorphs in SSS dispersion at different storage temperature over time (SSS = tristearin)

Storage temperature [°C]	Time [d]	$\bar{x}_{1,2}$ * [μm]	Turbidity* [NTU (ratio)]	$\Delta H_{m(\alpha)}$ [%]	$\Delta H_{m(\beta)}$ [%]
20	1.7	0.1 ± 0.00	441 ± 6.3	24.9/ 30.3	75.1/ 69.7
	7	0.1 ± 0.01	432 ± 13.4	18.2/ 19.6	81.8/ 80.4
	14	0.1 ± 0.01	437 ± 21.2	15.7/ 9.8	84.3/ 90.2
	28	0.1 ± 0.00	412 ± 17.0	7.8/ 9.3	92.2/ 90.7
	56	0.7 ± 0.71	n.d.	0.0/ 0.0	100/ 100
4	1.7	0.1 ± 0.00	444 ± 13.7	29.3/ 27.4	70.7/ 72.3
	7	0.1 ± 0.00	432 ± 15.8	27.1/ 24.8	72.9/ 75.2
	14	0.1 ± 0.00	439 ± 9.4	25.9/ 28.0	74.1/ 72.0
	28	0.1 ± 0.00	444 ± 17.0	26.0/ 27.6	74.0/ 72.4
	56	0.1 ± 0.00	464 ± 3.5	26.8/ 25.0	73.2/ 75.0

* $\pm$ SD for n = 6

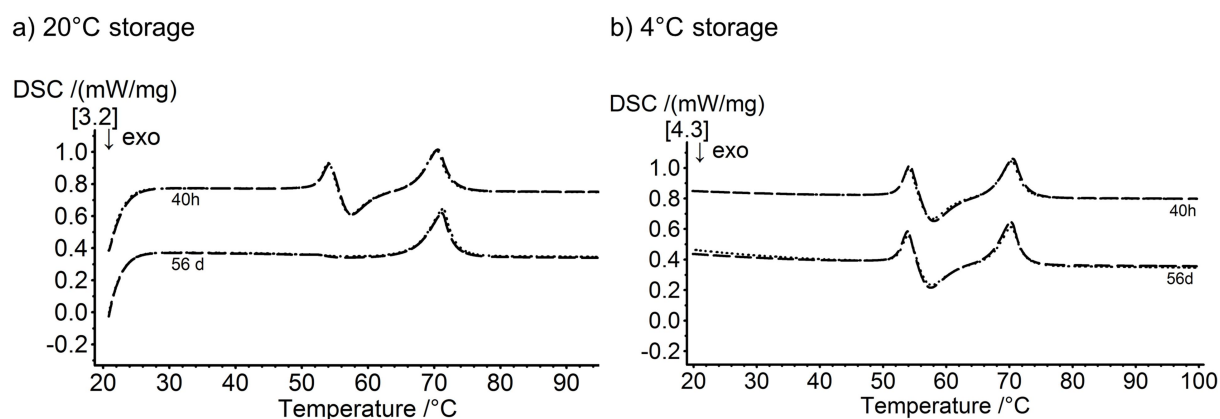


Figure 5-7: Polymorphic transition of SSS crystals in dispersion over time (a) at 20 °C storage temperature and (b) at 4 °C storage temperature (SSS = tristearin). Samples were heated from 20 °C or 4 °C, respectively, to 100 °C with a heating rate of 5 °C/min, and after 5 min. isothermal alignment cooled down to 0 °C with a cooling rate of 5 °C/min

liquid anymore nor dissolves lump-free in water. Since the cloud did not dissolve upon dilution turbidity could not be determined. Analyzing the DSC results it becomes obvious that in the dispersion initially a metastable state consisting of α - and β -crystals developed (Figure 5-7a). Over time a change from α - to β -crystals can be observed with the ratio decreasing from 25 % α -crystals in the dispersion to a system with only β -crystals after eight weeks of storage (Table 5-4). DSC results are in line with the polymorphic transition and associated gelation or aggregation as described by Arima et al. (2007), Awad et al. (2009), Munk et al. (2013), Westesen & Siekmann (1997). The molecular rearrangement increases the specific interfacial area, as the particle shape changes from the spherical α -polymorph to the platelet-like β -conformation (Dickinson et al., 1993; McClements, 2012b; Westesen & Siekmann, 1997). A possible approach to inhibit re-crystallization may be to cover the newly created interfacial area with additional surfactant, which is added to the dispersion after its preparation (Salminen et al., 2014a). In the present study, the same dispersion stored at 4 °C maintains its polymorphic state and remains liquid (Figure 5-7b). Physical stability of SSS dispersions during cold storage has also been reported by (Bunjjes & Koch, 2005). Since the free energy of the lipid crystals is lower at lower temperature, this can be attributed to the activation energy needed to initiate the polymorphic transformation (McClements, 2012b).

5.6. Conclusion

The aim of this study was to analyze whether solidification of lipophilic material at the oil-water interface or the oil phase has an impact on turbidity of a beverage cloud. An insufficient degree of crystallization of HME is most likely responsible for a lack of an effect on turbidity. Replacing the liquid oil phase with a lipid phase with high melting point led to an increase in turbidity by 50 %. The sample with SFC = 36% was not significantly more turbid but the sample with SFC = 54%. Thus, the positive effect of a solid lipid phase on the refractive index difference, and respectively turbidity, requires a critical SFC, SFC_{crit} of the dispersed phase. Further research is needed in order to determine SFC_{crit} more precisely. Physical stability upon storage depended on the temperature. DSC studies showed that the initial polymorphic structure of α - and β -polymorphs may undergo phase transition to only β -crystals making the dispersion useless. Since the metastable system is susceptible towards temperature fluctuations, further investigations are needed to improve the stability of the dispersion over a wide range of temperature. A very similar field of research struggling with recrystallization are SLNs initially invented as delivery system for fat-soluble drugs (Siekman & Westesen, 1994). Intensive studies are undertaken to stabilize SLNs either by inhibiting polymorphic transformation by finding suitable emulsifiers (Joseph et al., 2015) or to immediately crystallize in the most stable β -conformation (Schoenitz et al., 2014). Since the latter may not be obtained in the crystallization step but needs lengthy energy intensive thermal treatment and requires the investment in new equipment, future research of the interfacial interactions of emulsifier and crystallizing lipids is attractive for the food and pharmaceutical industry. Thus, more fundamental research on the interaction of food grade emulsifiers with the lipids at the interface is required to tailor solid structures with high kinetic stability.

5.7. Conflict of interests

The authors declare that they have no conflict of interest.

5.8. Supplementary material: Re-designing clouds to increase turbidity in beverage emulsions

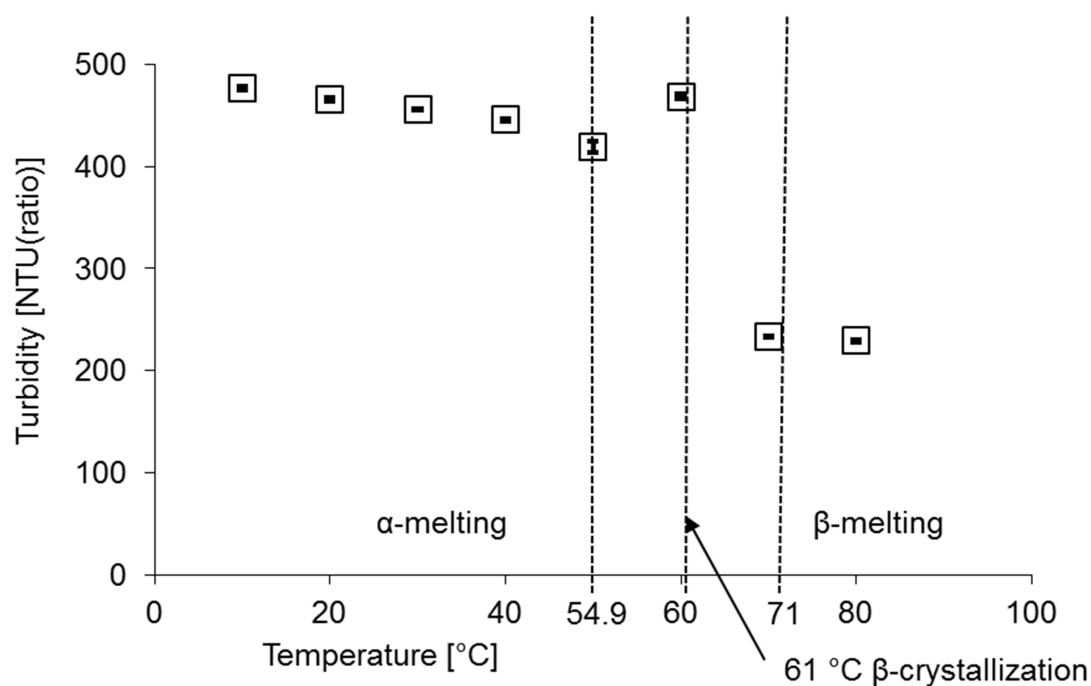


Figure 5-8: Turbidity depending on temperature for 0.2 % concentration of tristearin dispersion with one hour annealing time for each measured temperature. All data are means $\pm$ standard deviation for $n = 3$ (β -crystallization temperature taken from Da Silva, E., Bresson, S., & Rousseau, D. (2009). *Chemistry and physics of lipids*, 157, 113–119; α – and β – melting temperature result from DSC experiments (T_{mPeak1} and T_{mPeak2} , see Table 3)).

6. General Discussion

The aim of the present study was to understand the factors affecting turbidity in beverage clouds. The strategy followed to reach that goal is outlined in Figure 6-1. First, a correlation between instrumental turbidity readings and human visual perception was needed. The ability to judge an increase in turbidity successfully was of major concern (Manuscript I, chapter 2). The evaluation made it possible to decide whether a change in formulation significantly changes the perception of turbidity in the final application. These results were used throughout the present work to evaluate clouds.

Research on beverage emulsions typically focused on the kinetic stability of the emulsions. Therefore, a systematic approach to fully understand the factors influencing turbidity was needed. Great care was taken to keep all but one parameter constant to be able to judge the quantitative impact of individual factors (Manuscript I, chapter 2). The refractive index, more precisely the refractive index difference between dispersed and continuous phase had a significant impact on turbidity and might be tailored towards the desired properties of a cloud system. Furthermore, the decision was made to manipulate the interface and the dispersed phase.

The idea was to modify the interface with solid particles instead of conventional emulsifiers. The possibilities and limitations of particle stabilized emulsions, so-called Pickering emulsions, are extensively reviewed in Manuscript II, chapter 3. Another approach to modify the interface was to use a HME which solidifies upon cooling after a hot homogenization which is part of Manuscript IV (chapter 5).

The dispersed phase was modified by exchanging the typically dispersed oil phase based by a HML (Manuscript IV, chapter 5). The main challenge was to determine to what extent the lipids crystallized inside the dispersed droplets. The method developed to measure quantitatively the SFC of dispersions is described in Manuscript III (chapter 4). Following, the results are discussed with respect to the initially proposed hypotheses.

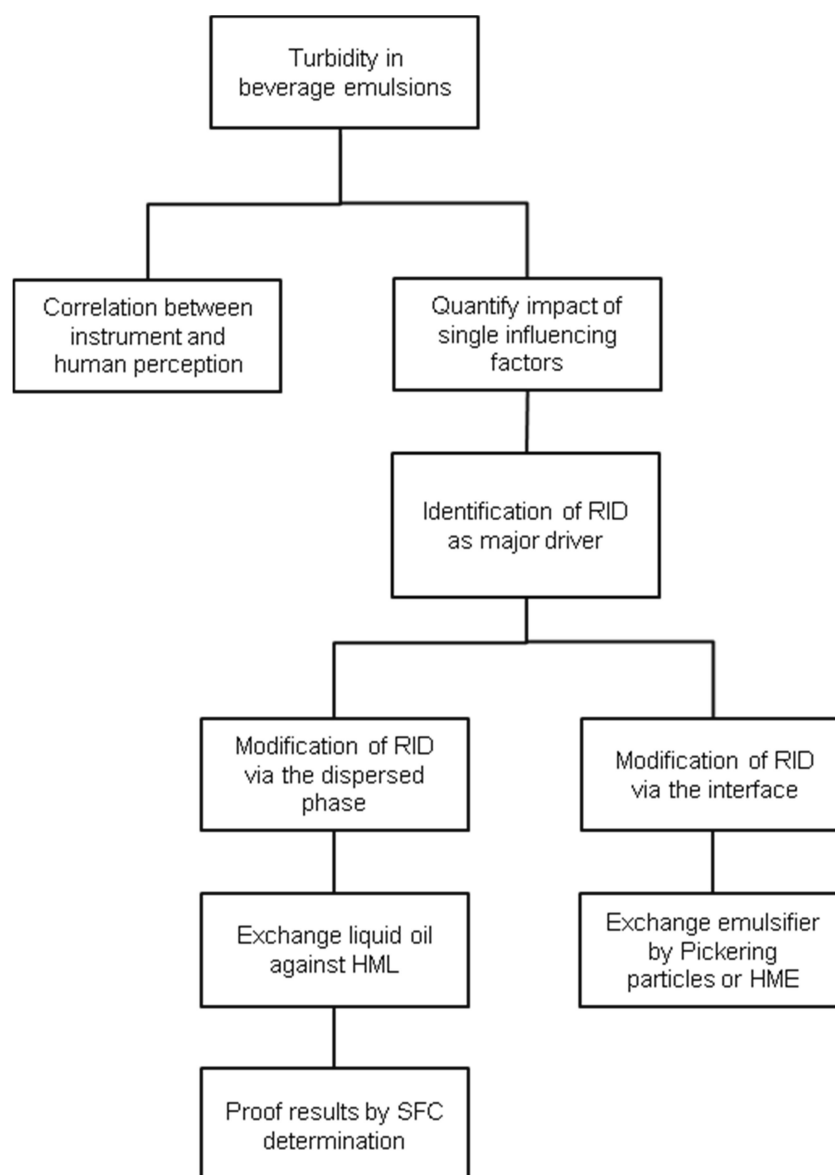


Figure 6-1: Strategy to increase turbidity in beverage emulsions (RID = refractive index difference; HML = high melting lipid; HME = high melting emulsifier; SFC = solid fat content).

6.1. Human visual perception of turbidity

The sensory evaluation to identify significant differences in turbidity was part of Manuscript I and was conducted via ranking methodology according to DIN ISO 8587:2010-08. The results were analyzed with the least significant difference test. The alignment of turbidity readings and the human perception was achieved for low [100 – 250 NTU (ratio)], medium [930 – 1500 NTU (ratio)] and high turbidity [4000 – 9000 NTU (ratio)]. Threshold determinations showed for

polystyrene beads in the size range of emulsion droplets (0.15 – 0.7 μm) that turbidity is perceived by humans starting from turbidity levels below 1 NTU under controlled viewing conditions (source of light, background, etc.) (Fleet & Siebert, 2005). Horne et al. (2001) found slightly higher values (3.5 NTU) for apple juice under less controlled viewing condition still demonstrating that humans are sensitive to detect turbidity. Thus, the examined low turbidity range representing beverage applications is well above the threshold and should be perceived as considerable turbid. Human response to different levels of intensity of certain stimuli has traditionally been conducted by magnitude estimation and belongs to the field of psychophysics. The most prevalent psychophysical law is Stevens Power law describing an exponential relationship between stimulus and human response (Stevens, 1957). Therefore, it seems likely that the human response to a visual stimulus like turbidity would as well follow a non-linear relationship. As can be seen in Figure 2-6, for the present emulsion a polynomial relationship was established. In the turbidity regimen of a typical cloud-based application between 100 - 250 NTU (ratio) the human response is most sensitive and 25 – 50 turbidity units difference are perceived as significantly different. Carrasco & Siebert (1999) concluded from a magnitude estimation study with polystyrene microspheres comparable to the size of the oil droplet size in beverage emulsions that the panelist response became saturated at turbidity values above 500 NTU (Carrasco & Siebert, 1999). In other cases like loudness or sweetness saturation effects were also observed (Zwislocki, 2009). This might be explained by the experimental design. Magnitude estimation requires the panelist to provide the turbidity values themselves with only one reference value and the intensity increase in turbidity is held constant by the factor of two between samples. In contrast with the ranking test in the present study no saturation threshold was detected. Across the whole measurement range of the turbidimeter people could rank the samples steadily. Samples were compared directly with each other in the ranking test and the turbidity differences were adjusted until the samples were perceived as different. This means as well, that even at the high turbidity range where 2000 NTU (ratio) were needed to differentiate samples it is still necessary to measure and compare turbidity during product development.

Summarizing, the sensory analysis confirms the hypothesis that the human perception differs from the linear concentration calibrated instrumental readings. A relationship was established between instrument and human perception enabling to judge beverages as significantly more turbid. The results of this correlation provide a new dimension to cloud development. So long

clouds, especially reformulations, had to match certain turbidity values. Now there is the possibility to confidently state whether there will be significant changes in the perception of turbidity in the final application.

6.2. Factors influencing turbidity

As outlined in the introduction, parameters affecting turbidity are oil droplet size, oil concentration and refractive index difference (RID) between dispersed and continuous phase (Mie, 1908; Oster, 1948; Kerker, 1969). Still, for complex polydisperse systems, like beverage emulsions, turbidity is poorly understood (Zhang & Reineccius, 2016). One reason might be that research on beverage emulsions mainly focused on kinetic stability. Few studies include turbidity in the qualitative evaluation of a beverage emulsion (Chantrapornchai et al., 1998; Chantrapornchai et al., 2001; Mirhosseini et al., 2008b; Mirhosseini et al., 2008c; Mirhosseini et al., 2009; Mirhosseini & Tan, 2010). Mirhosseini et al. (2008b, 2009, 2010) used response surface modelling to characterize and optimize orange oil beverage emulsion stabilized by gum arabic and xanthan gum using a central composite design. The aim was to optimize the formulation regarding turbidity, oil droplet size, polydispersity and density. Therefore, the results compromise four variables. A systematic advancement to quantify the single factors affecting turbidity was missing. Following, the results for the impact of oil droplet size, oil concentration and RID on turbidity are discussed.

In the present work, an exponential relationship between droplet size and turbidity was found (see Figure 2-1). Furthermore between the two smallest mean droplet sizes ($\bar{x}_{1,2}$) the scattering efficiency does not increase anymore around 0.2 μm . This is in line with previous studies where maximum turbidity was found for mean droplet size of 0.3 μm for orange oil beverage emulsions and 0.6 μm for latex particles (Hernandez & Baker, 1991; Hernandez et al., 1991). The differences are still in the order of the same magnitude and might arise from different methods and/or instruments for particle sizing and turbidity measurement. The findings are comparable to the literature approaching the scattering of light theoretically. Calculated with the Maxwell equations the droplet diameter inducing maximum scattering efficiency is calculated to be about equal in size to the wavelength of the light (Oster, 1948) indicating for even smaller droplets turbidity should decrease again. This was demonstrated by Zhang & Reineccius (2016) for mean

oil droplet sizes ranging from 0.09 – 0.5 μm . Therefore, it can be concluded that for the present emulsion systems the optimal surface weighted mean droplet size for maximum scattering is 0.2 μm . Presuming that due to good manufacturing practice emulsions are produced with an upper droplet size limit with $x_{90,3} < 1 \mu\text{m}$, the turbidity increased from 220 NTU (ratio) to 250 NTU (ratio) (values derived from Table 2-2 and Figure 2-1). Therefore, an optimization of droplet size can increase turbidity by maximal 20 %. In this turbidity range at least 50 NTU (ratio) are needed for a significant difference in turbidity (see Figure 2-5). Therefore, droplet size is not considered suitable to significantly change the human perception of the product.

For droplet concentration and turbidity a linear correlation is found (Figure 2-2). This is not per se a new finding and has already often been considered as a strategy to increase turbidity (Buffo et al., 2001; Almeida et al., 2015; Gharibzahedi et al., 2012; Jacome-Guth et al., 1991). However, at high oil content kinetic stability becomes an issue due to the used raw materials. GA is the most common emulsifier for beverage emulsions since it is able to stabilize submicron droplets in the acidic conditions ($\text{pH} \leq 3$) present in beverages (Dickinson, 2003). It is needed in very high emulsifier-to-oil ratios (1:1 and higher) and is used to full capacity. Essential oils like orange oil (846 kg/m^3) and 10 °Brix sugar solution (1047 kg/m^3) differ remarkably in density. Therefore, the oil phase is typically adjusted in density by weighting agents which are restricted in use. The most widely used glycerol ester of wood rosin, also known as glycerol abietate or ester gum (EG), is restricted to 100 ppm in the final beverage in US and EU (US Food and Drug Administration, 2002; Europäisches Parlament und Rat der Europäischen Union, 2008) which is already exhausted in modern formulations. Consequently the oil concentration is limited by food additive regulations and the capability of the emulsifier. Therefore, the parameter droplet concentration showed only potential for optimization but not to significantly increase turbidity.

A change in RID has the most pronounced effect on the optical properties of a beverage emulsion. The addition of 30 % weighting agent (glycerol ester of wood rosin, EG) increases the RI of the dispersed phase by 0.02. A positive effect of EG on beverage turbidity was also found by Kaufman & Garti (1984). Turbidity was increased by 72 % upon increase by 0.04 of the refractive index (RI) of the dispersed phase (see Table 2.4). In the same way it was shown that a decrease in RID caused turbidity to decrease. With increasing glycerol content of the aqueous phase the refractive index increased and turbidity decreased until the emulsions were clear when the RIs were identical ($\text{RID} = 0$) (Chantrapornchai et al., 2001). In the present study, the turbidity

difference is 115 NTU (ratio) at the beverage relevant low turbidity range where 50 NTU (ratio) are perceived as significantly different. Therefore, increasing the RID appears to be a suitable approach to increase turbidity in oil-in-water-emulsions. As outlined above addition of more EG is questionable directing to search for alternatives. Options to increase the RID are the modification of the continuous phase, the dispersed phase or the interface. Generally, the RI of water is lower than the RI of oil. Thus, a modification of the continuous phase would mean decreasing the RI of the continuous phase which is water. But, typical ingredients dissolved in the continuous phase like citric acid and sugar rather increase the RI of the continuous phase (Benitez et al., 2007b; Shachman, 2004a). Another approach would be to stabilize suspended particles in water and generating a turbidity system where dispersed and emulsified parts contribute to the scattering efficiency. The continuous phase of the emulsion will not determine the turbidity of the final beverage but the continuous phase of the final application. The composition of the final application will be chosen by the beverage manufacturer. Therefore the manipulation of the continuous phase was not judged a suitable strategy. Consequently modification of the interface and modification of the dispersed phase were chosen for further studies.

In conclusion, the second hypothesis was successfully confirmed by the results of the first manuscript. The RID between the phases of an emulsion was identified as the most promising factor influencing turbidity. Considering the possibilities led to the decision to modify the interface or the dispersed phase to increase RID in emulsions. The results of these two approaches are discussed in more detail below.

6.3. Increasing RI of interface

It is generally known that the RI of a material increases when the state changes from liquid to solid. A substance is in the state of highest order in the crystalline form. In a crystal lattice the phase relations are fixed, which leads to stronger external and internal interference of the light with the material compared to a liquid (Giacovazzo, 2005). Increasing the RI of the interface was approached via Pickering particles and HME.

6.3.1. Pickering particle

Solid particles instead of typical emulsifiers were reviewed regarding their ability to stabilize emulsions. Particle stabilized emulsions, so-called Pickering emulsions, gained huge interest recently due to their superior stability against coalescence (Binks & Horozov, 2006). As shown in Figure 3-1 the interface is stabilized by particles not soluble in either liquid phase (Binks & Clint, 2002). In dependence on the wetting properties and the resulting contact angle the particles immerse preferentially into one phase and can therefore form oil-in-water as well as water-in-oil emulsions (Binks, 2002). In terms of turbidity pigments or minerals with a very high refractive index like titanium dioxide (RI of 2.4) are promising particles to significantly modify the RID of the interface. A dense particle layer at the interface should therefore be able to increase turbidity. From the conclusions gathered in the detailed review of the stabilizing mechanisms underlying Pickering emulsions (see Manuscript II, chapter 3) can be drawn that long term stability in Pickering emulsions could only be achieved in systems forming a 3D-network throughout the sample (Drelich et al., 2010; Lan et al., 2007; Reger & Hoffmann, 2012). Network formation depends on the phase volume ratio between continuous and dispersed phase and particle concentration (Liu & Tang, 2013; Juárez & Whitby, 2012). Many studies showed that particle coverage and thus droplet stabilization was enhanced (Frith et al., 2008; Braisch et al., 2009; Binks et al., 2007; Binks & Rodrigues, 2007a; Binks & Rodrigues, 2007b) or only possible for slightly aggregated particles (Juárez & Whitby, 2012; Whitby et al., 2011; Nesterenko et al., 2014; Lan et al., 2007). Consequently, aggregation is not limited to the neighboring particles at the droplet surface but as well to particles of other droplets. However, such systems had a strong tendency to aggregate and consequently cream or sediment. This is caused by insufficient electrostatic repulsion of the particles. Apparently detrimental for the overall stabilization of the emulsion, this is indispensable for the local stabilization of individual droplets. In beverage systems, which will be diluted in the final processing step, the equilibrium between water, oil and particles will be destroyed leading inevitably to creaming or sedimentation.

Additionally, to form a stable emulsion the particles need to be at least one magnitude smaller than the droplets. Because of the high binding energy of the particles at the interface (Equation (3-2)), emulsions with large droplet size show high stability against coalescence. But oil droplets in an emulsion are found to be perceived as gritty starting from 4 – 7 μm (Kilcast & Clegg, 2002).

Consequently, particles small enough to stabilize emulsions with a droplet size distribution below this threshold, fall in the range of nanoparticles which are not accepted within the food sector.

6.3.2. High melting emulsifiers

Not dismissing the initial hypothesis, that solids at the interface might enhance turbidity, a further approach was investigated. Recently, Gupta & Rousseau (2012) and Kurukji et al. (2013) demonstrated that it is possible to produce emulsions with high melting emulsifiers which were solid at the interface at room temperature. Since the optical properties were not evaluated in the named studies, this led to the next approach. The aim was to investigate whether solidification of emulsifier at the interface after hot homogenization induces a higher turbidity in beverage emulsions. To achieve this, HMEs, namely PL, GSC and SSL, with melting temperatures well above room temperature (41 – 85 °C) were used to stabilize emulsions. The prepared emulsions had not been more turbid than the reference standard emulsion prepared with GA as emulsifier (Figure 5-1). The thermal analysis revealed no melting event for any HME emulsion. The emulsions are prepared with 1 % emulsifier. The sensitivity of DSC was tested for 1 % solids. Therefore, the case of total crystallization of the HME would have been recognized. In case of incomplete crystallization of HME sufficient sensitivity of DSC cannot be guaranteed. Since the RIs of the solid HME are higher than the RIs of the liquid HMEs or the oil phase (see Table 5-2), turbidity of the emulsions should rather be more turbid if the HME was solid at the interface. Therefore, the most plausible conclusion is that HME did not solidify at all or not to an extent affecting turbidity. In contrast to bulk, there will be a different thermodynamic equilibrium for each phase in the dispersed system changing the chemical potential for crystallisation (David & Klein, 2001). Additionally, as for Pickering particles, the packing density is constrained by the curved surface (see chapter 3.2.2). Assuming that the emulsifier initially forms a monolayer at the interface and needs to adapt to the curvature of the droplet, crystallization might be inhibited. Nevertheless, there are HME dispersions with a reported solid interface in the cited literature. But they prepared Pickering like emulsifier aggregates before emulsification (Kurukji et al., 2013; Pawlik et al., 2016; Gupta & Rousseau, 2012). While Kurukji et al. (2013) and Gupta & Rousseau (2012) used pure SSL respectively GSC, Pawlik et al. (2016) tuned hydrophobicity of the aggregates by combining tripalmitin with different emulsifiers like whey protein concentrate

(WPC) or lecithin. Those aggregates were 100 – 152 nm in size resulting in droplet sizes ranging from 2 - 10 μm . In these studies 2 – 8 % emulsifier were used for successful stabilization. Considering the results of the Pickering review and the size of the oil droplets, such systems are not considered suitable for the stabilization of beverage emulsions. Additionally, higher amounts of emulsifier contradict sustainability considerations.

Furthermore, turbidity of HME emulsions was significantly less turbid than the standard reference (Figure 5-1) indicating that the emulsifier has an impact on turbidity. Conflicting, in Manuscript I the influence of the emulsifier on the turbidity of a beverage emulsion was negligible at beverage relevant dosages (see Figure 2-4). Turbidity differences were investigated at 0.2 % and 2 % dosage for four types of emulsifier. GA was compared to whey protein concentrate (WPC), modified starch (MS) and Q. At 0.2 % dosage GA and OSA emulsions reached slightly but not significantly higher turbidity values. At 2 % dosage the emulsion stabilized with the emulsifier with the lowest molecular weight (Q) was the least turbid compared to GA standard but not significantly less turbid like the HME emulsions. Since every molecule acts as a scattering center (Van De Hulst, 1981) this is attributed to the high molecular weight of GA only taking into account at higher dosage levels. Looking at the formulation of HME and GA emulsions (Table 5-1) it becomes obvious that the required quantity of GA is significantly higher than that of the HMEs (13.5 % compared to 1 %). Therefore it must be concluded that high molecular weight polymers add to the scattering efficiency of an emulsion. In theory an emulsifier diffuses to the interface and adopts the conformation that minimizes its free energy while a dynamic exchange of emulsifier molecules is possible. It remains unclear though if the scattering happens at the interface or in the continuous phase. However, since beverage emulsions are typically stabilized by high molecular weight polymers, the emulsifier does not present an opportunity to increase turbidity.

The initial hypothesis, that the scattering efficiency of an emulsion can be increased via the interface, could not be verified with HMEs. Indeed, emulsions with PL, GSC or SSL as emulsifier were significantly less turbid than the GA reference. This is mainly attributed to the fact that no crystallization could be demonstrated. The chemical potential in the dispersed system will be different from bulk and might impair crystallization.

6.4. Increasing RI of dispersed phase

The third hypothesis claims that the solidification of the dispersed phase increases the RID of a dispersed system. Helgasson et al. (2015) observed increased turbidity upon solidification of lipids in a dispersion supporting the hypothesis. For octadecane emulsions with comparable oil droplet distribution to the present dispersions absorbance readings increased by 140 % upon solidification of the dispersed phase. The solid state of the lipids was dependent on storage temperature and proven by DSC. However, absorbance readings cannot be translated into the turbidity units used in the present study. The results in Manuscript IV showed that the use of HML instead of oil increased turbidity successfully by 50 % (Figure 5-2). Saturated or hardened lipids, namely HPK, PPK, HPF and SSS; with increasing melting point (39.5 °C, 45.5 °C, 59.7 °C and 74.7 °C respectively) were chosen to investigate whether turbidity increases upon crystallization of the dispersed phase. In bulk the SFC of the chosen lipids was ≥ 96 % and the RI was 0.05-0.08 higher compared to liquid. But, no direct correlation to the melting point could be established. This can be attributed to the remarkable differences between crystallization of dispersed lipids compared to the crystallization in bulk (Povey, 2001). The extent of differences occurring in surface-to-volume ratio and increase in droplet number as a result of the size reduction of the lipid phase has recently been highlighted (McClements, 2012b). Due to the reduced size and the increased surface the total lattice energy needed to melt the lipid is smaller than for a large bulk quantity. Considering a perfect cubic crystal (Kossel model) tiny growth units can be differentiated according to their interaction energy depending on the neighboring growth units (Aquilano & Sgualdino, 2001). In dispersed lipids quantitatively more edge-sides (no neighboring growth unit) exist which spend less energy to be removed from the crystal lattice. Accordingly, a size dependency for the melting temperature in dispersed lipids was found which is lower than the bulk melting temperature (Unruh et al., 1999). Skoda & van den Tempel, (1963) found the same dependency but attributed this to the increase in droplet number with reduced size. In the present samples the above mentioned decrease in melting temperature ranged from 2 – 7 °C. The difference in melting temperature increases with decreasing initial bulk melting temperature (see Table 6-1). For the sample with the highest melting point, SSS, ΔT_m increases again. This might be attributed to polymorphism which will be discussed in more detail in chapter 6.5. Considering the curved interface of the droplet and the cubic form of crystals the question arises whether dispersed lipids can fully crystallize. Additionally, the hydrophobic parts

Table 6-1: Melting temperatures T_m [°C] of bulk compared to dispersed lipids as means of $n = 2$. hydrogenated palm kernel fat (HPK), palm & palm kernel fat (PPK), 391 hydrogenated palm fat (HPF) and tristearin (SSS).

Lipid	Bulk T_m [°C]	Dispersed T_m [°C]	ΔT_m [°C]
HPK	39.1	32.0	7.1
PPK	45.5	39.0	6.5
HPF	61.1	58.6	2.5
SSS	74.6	70.5	4.1

of the emulsifier may protrude into the dispersed phase and have an impact on the structural arrangements in the droplet. Sophisticated methods are employed to elucidate nucleation and crystal growth in dispersed fats (Kalnin, 2009; Arima et al., 2009; Attama & Müller-Goymann, 2007). Surprisingly, no study was found which explicitly examines the degree of crystallinity in dispersed lipids in a comparable system. Consequently, a method is required to quantitatively determine the SFC in order to be able to attribute changes in turbidity to a crystalline dispersed phase.

In summary, it was demonstrated that the RID and respectively turbidity can significantly be increased by exchanging the dispersed phase with solid lipids. Crystallization of lipids in dispersed droplets is complex due to the confined space. Thus, a reduction in melting temperature and different polymorphic forms were observed. It is hypothesized that emulsified lipids do not recrystallize fully. To conclude that the increase in turbidity can be attributed to solids present in the dispersed phase a method for the quantification of SFC needs to be established.

6.5. Solid fat content determination in dispersions

The degree of crystallinity in dispersed lipids is also interesting for other fields of research, e.g. in SLPs for pharmaceutical applications. Attempts were undertaken to calculate a crystallinity index from DSC results (Schubert & Müller-Goymann, 2005; Seetapan et al., 2010; Keck et al., 2014; Attama et al., 2006; Kovačević et al., 2014). However, the thermal response of the sample is

depended on the tempering process, i.e. the chosen time/temperature profile (Walker & Bosin, 1971). Kalnin (2004) concluded that DSC recordings have two self-excluding limitations, resolution and sensitivity. Fast cooling rates provide sensitivity but poor resolution and vice versa (Kalnin et al., 2004). Additionally, the integration of a thermogram from an endothermic process is correlated to the melting enthalpy, the mass of the sample and its heat capacity (Le Botlan & Ouguerram, 1997). Since heat capacity differs for different polymorphic forms this seems not appropriate for the present samples. So far, there is no fast and reliable method to quantitatively describe the SFC in samples with finely dispersed lipid droplets in representative quantities and suitable for routine quality control. In this context a major advantage of TD-NMR would be a nondestructive, user friendly solution (Voda & van Duynhoven, 2009; Duval et al., 2006). Standard methods for SFC measurement of oil, spreads and seeds are readily available (DIN EN ISO 8292-1:2010-08:, 2010b; DIN EN ISO 8292-2:2008:, 2010b). Conventional SFC determination with TD-NMR is applicable for samples with a moisture content of 10 – 15 % (Todt et al., 2006). Samples with high amounts of free water cannot be treated this way. The NMR signal from water will interfere with the oil signal (Todt et al., 2006). Duval et al. (2006) approached this phenomenon using transverse relaxation decay deconvolution (TRDD) for protein stabilized emulsions with 30 % dispersed phase. To assign different mathematical models to the protons in the system, several assumptions have to be made concerning their mobility and their proton density increasing potential sources of error. Since in the present samples the emulsifier adds more protons to the system than the dispersed phase, the first approach was to eliminate the surplus signal. To be able to customize the method for samples with high water content the measuring principle needs to be fully understood.

Nuclear magnetic resonance can be applied to all nuclei exhibiting an intrinsic angular magnetic momentum so called spin. The small magnetic moment arises from the electric charges of the spinning nuclei. When placed in an external magnetic field B_0 these spins will line up comparable to a compass needle. According to quantum mechanics, for nuclei with a spin quantum number of $I = \frac{1}{2}$ (like for H protons) there are two possible energy states which result in two orientations: ‘spin up’ and ‘spin down’. The spins will precess around the magnetic field like a spinning top and the vectors describing the direction of the spins can be envisaged like a double cone orientated along B_0 (see Figure 6-2). Depending on the temperature the energetically more favorable energy states will be occupied more often (Hemminga 1992). The frequency of the

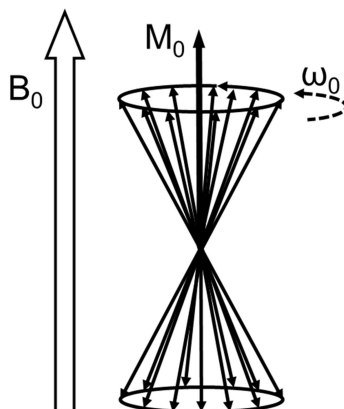


Figure 6-2: Orientation of spins precessing with Larmor frequency (ω_0) around B_0 ; adopted from Hemminga (1992): *Introduction to NMR*. Trends in Food Science & Technology, 3,p. 183.).

precession is called Larmor frequency ω_0 . By applying electromagnetic radiation of that frequency there will be a resonance causing an excitation of the nucleus to another energy state. The result is an oscillating magnetic field that induces an alternating voltage which can be measured as a function of time (Rudi et al. 2008). The energy quantum needed for a transition depends on the external magnetic field and the numerous interactions of the nucleus with its neighboring electrons and nuclei. This causes every nucleus to have a different effective resonance frequency and therefore enables spectroscopic measurements. A nucleus has as many resonance frequencies as the neighboring nuclei spin orientations (Van Putte & Van den Enden, 1974). The sum of all nuclei results in a macroscopic magnetization M_0 along the z-axis as shown in Figure 6-3a. After a 90° pulse along the x' -axis, M_0 is deflected towards y' (see Figure 6-3b). Subsequently the system regains equilibrium by relaxation. Mainly two relaxation processes take place. The longitudinal or spin-lattice relaxation with relaxation time T_1 describes the return of the system to equilibrium by emitting energy to the surroundings. The transversal or spin-spin relaxation with relaxation time T_2 is an entropic process describing the decay of the transverse spin magnetization caused by decoherence until the initial phase coherence of the nuclear spins is lost. The amplitude of the measured FID in TD-NMR is equivalent to the sum of all hydrogen protons in the sample and for water molecules relaxation is particularly slow (spin-lattice or T_1 relaxation at room temperature takes ~ 3 s). With inversion recovery the magnetization of the sample can be inverted by 180° pulse leading from an initial magnetization of $M_0 = 1$ to $M = -1$.

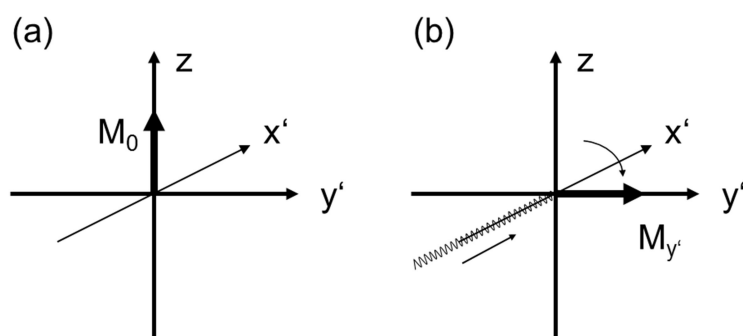


Figure 6-3: Macroscopic magnetization in equilibrium (a) and after a 90° pulse (b); adopted from Matissek, R. (2014): *Lebensmittelanalytik*. (5., vollst. überarb. Ausg.). Berlin: Springer Spektrum, p.93.

Depending on T_1 the protons of different origin will pass through a state of zero magnetization ($M = 0$) at different times while relaxation (Goudappel et al., 2001). At the point of zero magnetization the specific magnetization contribution to the measured signal is zero. Consequently for the present samples the point of zero magnetization of the continuous phase needed to be determined (see Figure 4-3). At an appropriately chosen ID the remaining magnetization of the dispersed phase can be exploited for further differentiation of signal contributions from solid and liquid components. Inversion recovery for emulsions was already successfully applied by Brosio et al. (1982) for triethanolamine stearate stabilized dispersions with 9 – 68 % lipid content. In the named study simultaneous determination of total water and lipid content is anticipated. But for the present samples the ratio between solid and liquid lipids of the dispersed phase is of interest. Another difference is the emulsifier used in the present study. GA type Senegal is a highly branched, long-chained polysaccharide consisting of up to ~ 90 % of arabinogalactan (Gashua et al., 2016). From the basic molecular formula of a carbohydrate ($nC_6H_{12}O_6$) can be derived that it consists to ~ 50 % of hydrogen. Since any hydrogen proton adds to the FID signal and GA is solubilized in water this is expected to have an impact on the mobility of the continuous phase and therefore the relaxation of water. Such effects were demonstrated for the relaxation of water molecules in starch solutions (Ritota et al., 2008; Hansen et al., 2009). Chemical exchange between water and starch molecules was observed. Additionally, GA has a proteinaceous fraction of about 5% which also affects T_1 relaxation as demonstrated by Lucas et al. (2005). For the present samples it is shown in Figure 4-4 that t_0 decreases

exponentially with increasing emulsifier concentration. The result indicates that the emulsifier considerably reduces the water relaxation. The optimal ID was with 0.5 s close to the ID of 100 % emulsifier solution (as used for dispersion preparation). The emulsifier is expected to diffuse to the interface and adopt the conformation that minimizes its free energy while a dynamic exchange of emulsifier molecules is not excluded. Biopolymers form relatively thick interfacial films with a fairly open structure (McClements, 1999). Derived from the relaxation behavior molecules within these films or parts of them are still highly mobile. The remaining FID after the inversion delay (Figure 4-5) was analyzed by extrapolation of the fast and slowly relaxing signals to the intercept. From the ratio of solid and liquid lipid the SFC was derived yielding good reproducibility (standard deviation: 0.5). Consequently, SFC evaluation of the dispersed lipids revealed increasing SFC with increasing melting point of the lipid (see Figure 5-3). Only two of the four dispersions with $\text{SFC} > 36\%$ were significantly more turbid than the reference emulsions. Thus, rather a minimum crystallinity is needed for a significantly more turbid dispersion. In Manuscript I an increase in RID of 0.04 due to the use of 30 % weighting agent led to an increase in turbidity of 70 % compared to 50 % more turbidity with the same increase in RID for the lipids. With 54 % and 65 % the SFC of the dispersed lipids differs remarkably from the SFC in the bulk lipids ($\text{SFC} \geq 98\%$) which might explain for the difference in measured and expected turbidity increase. Assuming the RI is the only difference affecting turbidity in the measured samples, with regard of the results from the impact of weighting agent the RID of HML was expected to cause more turbidity. Thus, the effective RI of the dispersed HML is lower than the RI of the bulk fat and will range somewhere between the RI for the liquid (1.45) and solid (1.52) lipid, most likely below the RI of the weighted orange oil phase (1.49).

Still, to allow a more detailed interpretation of the light scattering in the droplets more details of the structure inside the droplets is needed. Crystallization or rather nucleation is differentiated in heterogeneous and homogeneous (Aquilano & Sgualdino, 2001). Homogeneous nucleation starts from the bulk phase and extends outwards (Sato, 1999). This typically happens in pure bulk phases. Heterogeneous nucleation on the other side is promoted by surfaces or impurities which act as seed crystals (Dickinson et al., 1996; Kalnin et al., 2004). Surfaces can act as nucleation sides due to the contact angle between liquid lipid and surface reducing the free energy needed to start crystallization (Aquilano & Sgualdino, 2001). It is much discussed which type of crystallization is more likely to happen in dispersed lipids. One consideration takes into account

that for heterogeneous nucleation a seed crystal would be necessary in each droplet which is highly unlikely considering the magnitude of droplets at submicron droplet sizes (McClements, 2012b). On the other side the surface, which can act as nucleation side is highly increased in submicron droplets. In the present samples it remains unclear whether solid and liquid droplets are present or incomplete crystallized droplets. Assuming the first case a homogeneous nucleation would be suggested while in the second case heterogeneous nucleation is more likely. Future studies should expand the analytical methods to allow more detailed differentiation of the structure elucidation in dispersed systems as it is already demonstrated for bulk triglycerides like SSS by Transverse Relaxation Decay Deconvolution (TRDD) (Trezza et al., 2006).

It was demonstrated that determination of SFC in dispersed lipids was not readily available. It was possible to establish a method to determine the SFC in dispersion by combining two NMR measurement principles, inversion recovery and T_2 relaxation. The results revealed high mobility of GA in dispersion and that crystallization of dispersed fats, depending on the melting point of the lipid, is incomplete at room temperature. The findings help to elucidate the structure of HML dispersions and provide a fast and nondestructive method which can be routinely used in quality control for research and product development. To validate the method the next step should be to compare this approach by the TRDD-method of Duval et al. (2006). Additionally, future research to elucidate the structure of dispersed lipids would help to understand the scattering of HML-dispersions.

6.6. Stability of polymorphic dispersed phases

Besides turbidity, a critical aspect is the physical stability of emulsions, both in concentrated and diluted version (Dickinson 1994). Beverage industry requires an emulsion stability period of at least six months (Tse & Reineccius, 1997). Since an emulsion is a dispersion of two immiscible liquid phases, it is an a priori thermodynamically instable system. By applying enough mechanical energy to this system, e.g. by homogenization, a metastable condition, kinetic stability, can be achieved (McClements, 2005). Consequently, several types of instability can occur. As shown in Figure 6-4, emulsion instability mechanisms can be divided into flocculation, coalescence, Ostwald ripening and gravitational separation, namely creaming or sedimentation. Flocculation is a process caused by insufficient electrostatic or steric repulsion leading to

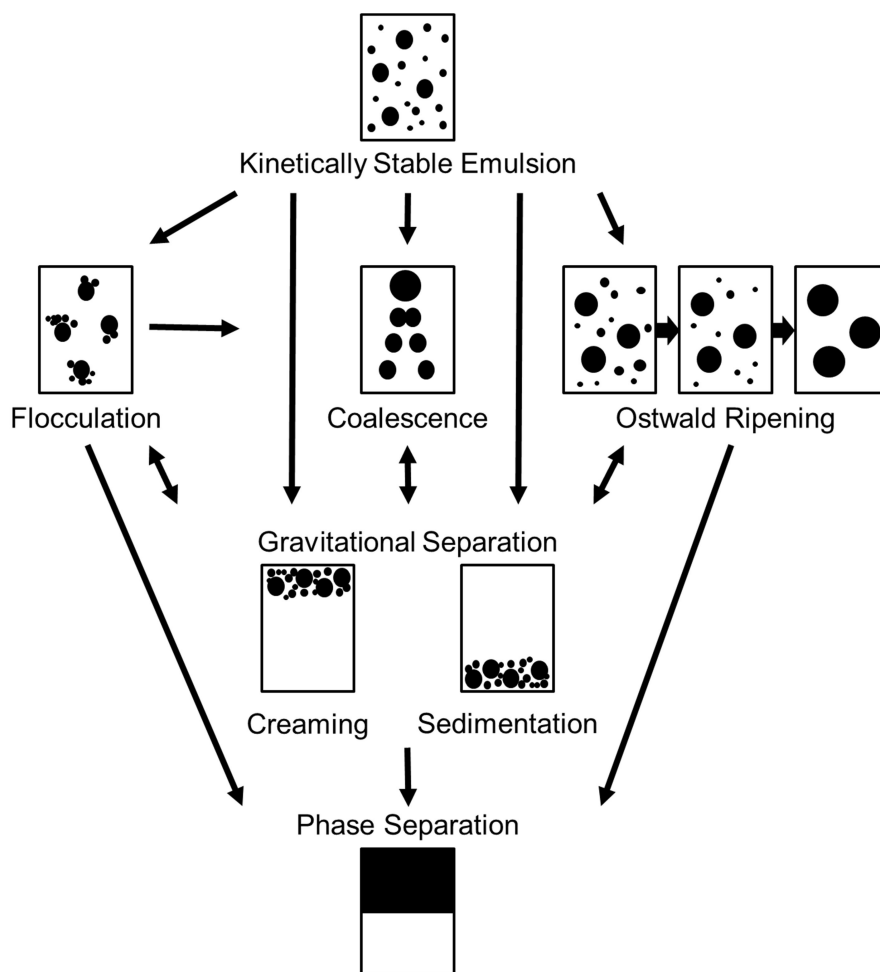


Figure 6-4: *Instability mechanisms occurring in emulsions (adapted from Lopetinsky, R. J., Masliyah, J. H., & Xu, Z. (2006): Solids-Stabilized Emulsions: A Review. In Bernard P. Binks, Tommy (edt.) Horozov (Eds.): Colloidal Particles at Liquid Interfaces: Cambridge University Press, p. 189. Copyright 2006 Cambridge University Press).*

aggregation of dispersed droplets (Tadros, 2013). Flocculated droplets keep their initial size and can be separated with mechanical force. Nevertheless, flocculation enhances gravitational separation like sedimentation or creaming (McClements, 2005). In beverage emulsion so-called depletion flocculation can occur when polymers compete for hydration due to steric forces (Jenkins, 1996). This was found to happen for mixtures of GA and modified starch, two commonly used emulsifiers for beverage emulsions (Chanamai & McClements, 2001).

Coalescence is the merging of droplets with an insufficiently stabilized interface caused by thin film rupture. Droplets reduce in number and increase in size until the surface area is decreased to an extent the emulsifier can stabilize. Inevitably the resulting bigger droplets also enhance the

gravitational forces applying to the system (Lopetinsky et al., 2006). The polymers used for stabilization typically form a film around the oil droplets. Because of this interfacial film, true coalescence of oil droplets is seldom seen in beverage emulsions (Stauffer, 1999).

The result of Ostwald ripening is very similar to coalescence yet the mechanism is different. Ostwald ripening is driven by the Laplace pressure and diffusion leading big droplets to grow on the cost of small droplets. Flavor containing oil phases are particularly prone to Ostwald ripening since individual flavor compounds can have considerable water solubility (Lim et al., 2011).

Gravitational separation is the most frequent cause of emulsion instability due to the most likely different densities of the two phases. The velocity of separation is described by Stokes' law in Equation (6-1):

$$(6-1) \quad v = \frac{2(\rho_2 - \rho_1)gr^2}{9\eta_1}$$

with v being the velocity, the density (ρ_1) and viscosity (η_1) of the continuous phase, the density (ρ_2) and droplet radius (r) of the dispersed phase and gravitational acceleration g (McClements, 2005). Depending on the positive or negative result, the system will tend to cream or sediment respectively. As can be derived from Equation (6-1), the squared droplet size has the biggest impact on the result. This makes droplet size distribution an important factor for quality control in beverage emulsion production. Therefore, homogenization conditions are typically chosen to keep $\bar{x}_{90,3}$ below 1 μm . However, ring formation on the neck of the bottle is a common defect observed in beverages containing emulsions (Reiner et al., 2010). While in many food emulsions viscosity plays an important role, in the highly diluted final beverage the viscosity of the continuous phase is very close to the one of water making beverage emulsions a unique class among food emulsions (Dickinson, 2010). The prepared HML-emulsions were stable for more than two month. Crystallized lipids have higher density than liquid lipids and should therefore have a positive effect on emulsion stability (McClements, 2005).

However, as mentioned above, polymorphism was found in the most turbid sample. Polymorphism is a phenomenon inherent to TAGs. Fatty acid chains are flexible considering molecular conformation and lateral packing (Kaneko, 2001). Therefore they can crystallize in different forms. As can be seen in Figure 6-5a fatty acid carbon chains arrange in a zigzag pattern.

Accordingly two black dots represent the end view of such a chain in the following parts of Figure 6-5. TAG crystals are stacked molecule layers. The Thickness of these layers is referred to

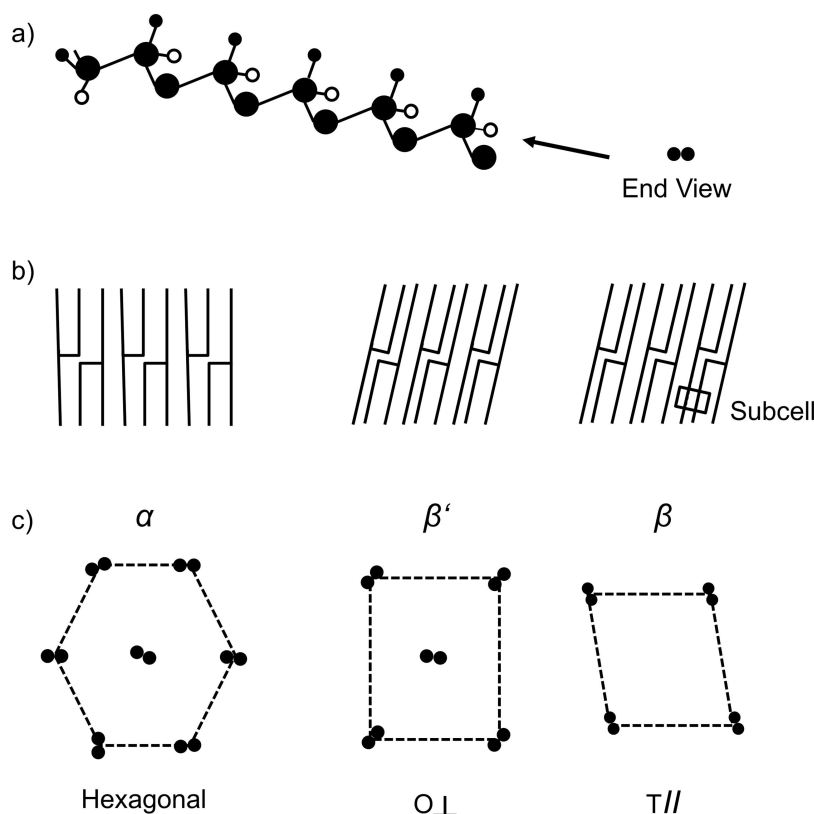


Figure 6-5: Polymorphism in triacylglycerols (TAGs) adapted from Lopez, C., Lesieur, P., Keller, G., & Ollivon, M. (2000): *Journal of colloid and interface science*, 229, 62–71. a) Planar zigzag conformation of hydrocarbon chains of saturated fatty acid as a 3D view along its main axis (most of the front hydrogen is not drawn for clarity). b) Three main types of TAG chain longitudinal packing for trisaturated TAGs (fatty acid chains are shown as straight lines). c) α -, β' -, and β -subcells of chain lateral packings; hexagonal (Hex.), orthorhombic perpendicular ($O\perp$), and triclinic parallel ($T\parallel$) (only carbon atoms are drawn as the end view above).

as longitudinal stacking or long-spacing (Lopez et al., 2000). In the present work only unsaturated or rather hardened lipids were used, which typically arrange in a double-chain-length structure (2L) as depicted in Figure 6-5b. The cross-sectional packing or short-spacing between the aliphatic chains are used to identify the crystalline subcells. The three main polymorphs associated with TAGs are α -, β' - (beta prime) and β -crystals (Timms, 1984). The differences are shown in Figure 6-5c. The α -polymorph has a hexagonal subcell, the β' -polymorph is

orthorhombic perpendicular ($O\perp$), and the β -crystals arrange in triclinic parallel ($T//$) subcells. In this case parallel means the zigzag of adjacent chains are in step whereas perpendicular means out of step (Himawan et al., 2006).

With the different dense packing structures the polymorphs differ in their respective Gibbs free energies (G) and therefore their stability towards transformation at constant temperature and pressure (Himawan et al., 2006). Polymorphism in TAGs is a monotropic phenomenon. Thus, a transformation from one crystal structure in the other occurs only from the least stable to the most stable conformation. Consequently, the polymorph with the highest free energy, the α -polymorph, is least stable, followed by the β' -form.

The β -polymorph is the one with the least free energy forming the most stable, large and plate-like crystals (Sato & Ueno, 2011). The crystal structure determines physical properties such as melting point, heat capacity and molecular flexibility (Sato & Ueno, 2001). In the present study, the SSS dispersion yielded the highest turbidity and was further investigated. This sample crystallizes in two polymorphic forms, α and β , with an additional melting event at 55.8 °C which is not the case for bulk SSS (see Figure 5-4). This means the a priori metastable system exhibits a second metastable condition. For SSS dispersion recrystallization leads to instability after two month stored at room temperature (Figure 5-4). Cold storage at 4 °C was able to inhibit that process. Reducing the thermal energy of the sample will enhance the kinetic stability but does not change the state of the system. Thus, strategies to circumvent the metastable condition in the dispersed phase need further research. Possibilities are either to arrest the transformation from α -to β -crystals or directly crystallize in the stable β -conformation. Crystallizing initially in β -conformation requires lengthy and energy intensive isothermal crystallization steps during processing and requires new equipment (Schoenitz et al., 2014). Therefore, a more feasible approach might be to find suitable particles or molecules which act as template at the interface promoting the growth of the anticipated crystal structure. Talc particles were found to promote the crystallization of bulk TAGs in stable polymorphs (Yoshikawa et al., 2015). However, the particles used were at least 0.6 μm in size leading for particles smaller than the dispersed droplets to the same skepticism as nano-sized Pickering particles. Nevertheless, the potential of foreign particles to act as templates was explained by considering the energy barriers for homogeneous and heterogeneous crystallization which are expressed by Equation (6-2) and (6-3):

$$(6-2) \quad \Delta G_{hom}^* = \frac{fv^2\gamma^3}{(\Delta\mu)^2}$$

$$(6-3) \quad \Delta G_{het}^* = \Delta G_{hom}^* \left(\frac{1}{2} - \frac{3}{4} \cos \theta + \frac{1}{4} \cos^3 \theta \right)$$

where, ΔG^* is an energy barrier to overcome for nucleation, $\Delta\mu$ is the Gibbs free energy difference between liquid and solid phase, f is a factor specific to the shape, v is a molecular volume in the nucleus, γ is an interfacial energy between the nucleus and the mother phase, and θ is the contact angle between nucleus and particle surface. According to Equation 3, small values for θ decrease ΔG^* . With higher molecular affinity the contact angle will decrease (Yoshikawa et al., 2015). Thus, fatty acid moieties should facilitate crystallization. It was demonstrated that the emulsifier has a pronounced effect on nucleation and crystallization in emulsions (Awad & Sato, 2001; Bunjes & Koch, 2005; Gülseren & Coupland, 2007; Salminen et al., 2014b; Salminen et al., 2014a). Bunjes & Koch (2005) for example found that saturated phospholipids promoted crystallization and slowed down polymorphic transition. Addition of highly hydrophobic sucrose oligoester (SOE) and highly hydrophilic SOE containing a palmitic acid moiety remarkably retarded crystallization-induced destabilization of oil-in-water emulsions (Arima et al., 2007). For hydrophobic emulsifiers with a long hydrophobic chain (propylene glycol monostearate, PGMS) was demonstrated that the crystallization temperature of emulsified triglycerides increased (Kalnin et al., 2004). Due to the lyo- and thermotropic and therefore partially structured properties of lipid based molecules it is assumed that the emulsifier will orient at a densely packed interface. Consequently, the structuring of dispersed lipids via suitable emulsifier is particularly interesting. In this regard, a substance accepted for use in beverages is octenyl succinic anhydride modified starch. Ideally its hydrocarbon side chains promote crystallization of stable polymorphs and also higher SFC rates to optimize turbidity. While lots of research is undertaken for dispersed fats emulsified with lipophilic emulsifiers like fatty acid ester ($HLB \leq 7$), no studies were found for the crystallization of biopolymer stabilized dispersed TAGs. To conduct these investigations further methods are needed to elucidate structural conformation and interactions between emulsifier and lipids at the interface. While DSC results were considered to depend on the time/temperature profile, TD-NMR methods need further development for the differentiation of polymorphs. Another method to investigate crystal structures and gain information on the mobility of individual components is based on off-magic angle spinning (MAS) (Mayer & Lukowski, 2000). Due to the proximity of molecules in crystals

strong interactions typically lead to line broadening making spectral resolution difficult to impossible for solids. Spinning the sample around the magic angle ($\theta_m = 54.74^\circ$) enables to measure crystal structures (Matissek, 2014). Well defined deviation from the magic angle reduces spectral resolution to a certain extent but still allows spectral assignments of chemical components. At the same time line shapes of the corresponding signals reflect dynamic parameters (Mayer, 1999). Unfortunately, the fast spinning at 1 – 4 kHz acts like a centrifuge on dispersion samples. Additionally, the comparable low dispersed phase volume (7 %) and the high carbon content of the emulsifier resulted in bad signal-to-noise ratios. Another approach to deepen the understanding of the present samples was high resolution ^1H -NMR which is a unique tool to study molecular interactions in solution. The same measuring principle applies as in TD-NMR but in stronger magnetic fields allowing for detailed spectral resolution. Several pulse sequences to suppress a water signal exist (Claridge, 2016). With complex studies combining the results of T_1 -, T_2 -, diffusion- (DOSY), 2D-HMQC- and LOGSY-experiments information on the location of the emulsifier (polyethylene glycol ester of stearic acid) and crystal arrangement in dispersed tripalmitin droplets was gained (Garcia-Fuentes et al., 2004). However, for the present samples the similarity of proton relaxation from polysaccharides and lipids complicates the analysis. Furthermore long range interactions between emulsifier and lipid are targeted instead of short range interactions inside a molecule which are typically investigated by NMR for structure elucidation. A comparable interaction is the protein-ligand binding in enzymes. A popular technique to investigate these interactions is via saturation transfer differences (STD) (Viegas et al., 2011). The STD-NMR experiment is based on the nuclear Overhauser effect (NOE) which arises from dipolar interaction between spins. Approaching dipoles attract or repel each other often dominating the relaxation process of neighboring protons resulting in incomplete relaxation between pulses (Claridge, 2016). Basically, an STD experiment is the subtracting of two spectra. In one spectrum the emulsifier is selectively saturated (*on-resonance* spectrum) obtained by irradiating at a region of the spectrum that contains only resonances with signal intensities I_{SAT} . The other one is recorded without emulsifier saturation (*off-resonance* spectrum), with signal intensities I_0 . In the difference spectrum ($I_{STD} = I_0 - I_{SAT}$) only the signals of the lipids that received saturation transfer from the emulsifier (via NOE) will remain. However, STD for the present samples resulted in overlapping signals which could not clearly be identified due to the chemical similarity between emulsifier and lipids. Consequently, further approaches need to be investigated. Future studies for structure elucidation on polymorphic transition and liquid

compartments in lipid droplets could be achieved by small (SAXS) and wide (WAXS) angle X-ray diffraction (Anantachaisilp et al., 2010). WAXS analysis can be used to elucidate the effect of liquid compartments on the subcell structures and polymorphism of the solid lipid, while SAXS can be used to investigate the interlayer spacing in the lamellar structure, which can be used to observe polymorphic transitions (Schubert et al., 2006). Another possibility is combined synchrotron X-ray diffraction analysis with simultaneous DSC studies delivering time-/temperature-resolved information on the transformational changes. This was used by Joseph et al. (2015) to investigate dispersed lipids. It could be demonstrated that polyvinyl alcohol increased stability of the α -polymorph significantly while ubiquinol (coenzyme 10) enhanced α - to β -transition (Joseph et al., 2015).

In conclusion, polymorphism leads to a metastable dispersed phase which requires further research. Crystallization might be directed with suitable emulsifiers and food-grade biopolymers are not yet investigated in this respect. The way emulsifiers self-organize at the interface is not fully understood due to a lack of suitable instrumentation (Kalnin et al., 2004). This would be crucial for the structure elucidation of SLPs. Future work should concentrate on methodical possibilities that account for the presence of solids and a high amount of water as well as the chemical similarity between polysaccharides and hydrocarbons.

7. Conclusions

The comprehensive experimental approach of the present thesis contributes to the understanding of turbidity in beverage emulsions. Thus, a basis is provided to tailor optical properties, to increase flexibility in product design by uncoupling flavor and optical appearance, and to develop more efficient and weighting agent free solutions. In more detail, sensory analysis proved that human visual perception of turbidity does not follow a linear but a polynomial correlation. Determination of the least significant difference revealed that these differences depend on the overall turbidity of the system and provide the opportunity to judge samples according to customer preferences. With specific limitations of the application, factors like droplet size and droplet concentration are not suitable for increasing turbidity of beverage emulsions, but the refractive index difference is a tunable factor with significant impact on turbidity. HML provided 50 % more turbidity in beverage emulsions than liquid oil and may serve as an alternative to the use of a weighting agent. A higher density of a solid lipid compared to a liquid lipid adds positively to the kinetic stability of dispersions. However, the dispersed solid lipid crystallizes in two polymorphic forms leading to a metastable state in an a priori metastable system.

From a commercial point of view the visual appearance of a food or beverage is crucial for customer acceptance (Mirhosseini & Tan, 2010). Therefore linkage of instrumental readings and sensory evaluation provides a powerful resource for predicting consumer reactions and accordingly customized product development. Furthermore the growing demand for sustainability and naturalness (Green et al., 2013) can be satisfied with more efficient and weighting agent-free solutions based on a dispersed phase in the solid state. On the other hand, beverage emulsions require long term stability in concentrated and diluted form (Reiner et al., 2010) and a metastable dispersed phase with low kinetic stability is not acceptable. Other sectors like pharmacy would also benefit from strategies to slow down polymorphic transition and to increase stability of these metastable systems. SLPs are of great interest for pharmaceutical applications as drug delivery systems (Svilenov & Tzachev, 2014), where non-targeted repulsion of the active ingredient from a lipid matrix is a severe issue. One strategy described in the literature is e.g. based on liquid compartments to avoid drug repulsion (Salminen et al., 2013). For beverage clouds this approach might impair the scattering efficiency due to less crystallinity. Therefore, direct crystallization in the stable β -conformation is one option to avoid instability of

the dispersed phase. However, so far β -crystallization was only successful by introducing time and energy demanding tempering steps in special equipment (Schoenitz et al., 2014) and is thus not cost-effective and contradicts the idea of sustainability.

It becomes obvious that future research must focus on an in-depth understanding of crystallization and polymorphic transition in dispersed systems and more specifically on the role of the emulsifier within these processes. Emulsifiers or particles may act as a template for β -crystallization. It is known that emulsifiers with long chain fatty acid moieties accelerate nucleation of lipids (Sato, 1999). Bunjes & Koch (2005) for example found that saturated phospholipids promoted crystallization but slowed down polymorphic transition. Addition of highly hydrophobic sucrose oligoester (SOE) and highly hydrophilic SOE containing a palmitic acid moiety remarkably retarded crystallization-induced destabilization of oil-in-water emulsions (Arima et al., 2007). In this regard, a substance accepted for use in beverages is octenyl succinic anhydride modified starch. Ideally they would as well promote higher SFC rates to optimize turbidity because of its hydrocarbon tails. In this context TD-NMR provides a nondestructive, fast and reliable possibility for process and quality control (Gianferri et al., 2007) and the method developed in the present study provides a tool facilitating research opportunities. Automation of the data evaluation would in this respect play an important role. Investigation of the interface is still limited due to the lack of suitable instrumentation. Complex NMR studies using diffusion 2D-experiments could provide information on the crystal arrangement in dispersed droplets (Garcia-Fuentes et al., 2004). However, the similarity of proton relaxation from polysaccharides and lipids complicate the analysis of food grade approaches by NMR. Further structure elucidation on polymorphic transition and liquid compartments could be achieved by small and wide angle X-ray diffraction (Anantachaisilp et al., 2010) and time-/temperature-resolved synchrotron X-ray diffraction analysis with simultaneous DSC studies (Joseph et al., 2015).

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Curriculum vitae

Professional Experience

Jan 2014 – Oct 2017	Technical University Berlin Research Assistant Supervision of bachelor and master students Organization and supervision of practical laboratory workshops
Oct 2012 – Nov 2013	Symrise AG, Singapore Development Engineer Optimization of spray drying formulas for reaction flavors Product development of in-base flavors for instant noodles
Feb 2012 – May 2012	Australian Wine Research Institute, Adelaide Research Assistant Research project: <i>Relationships between harvest time and grape anthocyanin and skin tannin concentration</i> Project work: <i>Influencing wine style through management of oxygen during Winemaking</i>
Feb 2011 – Aug 2011	Symrise AG, Holzminden Internship and diploma thesis Spray drying of flavor emulsions
Apr 2010 – Oct 2010	GDC/RDC Dry Foods, Unilever Deutschland GmbH, Heilbronn Internship in R&D Planning, execution, analysis and presentation of pilot plant trials Optimisation of drum drying process for vegetables
Sep 2004 – Aug 2005	Stammhaus – Gemeinschaft Unser Platz im Leben e. V., Cologne Voluntary work residential treatment of mentally and physically disabled people socio-paedagogical intervention

Education

- Jan 2014 – Oct 2017 **PhD (Dr.-Ing.)**
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- Oct 2005 – Feb 2012 **Diplom-Ingenieur**
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