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Abstract

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

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

1   Introduction

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

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

As a consequence, proteins and low molecular weight emulsifiers are frequently co-formulated and coexist in emulsions. The emulsifying constituents may also interact with each other and coexist at the interface with resulting change in interfacial behaviour. These interactions are based on hydrogen bonding, hydrophobic and

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

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

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

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

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

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

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

2   Materials and methods

2.1   Materials

For the preparation of spray-dried emulsions, whey protein isolate (WPI) (Lacprodan DI-9224, Arla Foods Ingredients Group P/S, Viby, Denmark), maltodextrin with a dextrose equivalent of 14 (C* Dry ™ MD 01910, Cargill Deutschland GmbH, Krefeld, Germany) and medium-chain-triglyceride oil (MCT-oil, WITARIX® MCT 60/40, IOI Oleo GmbH, Hamburg, Deutschland) were used. The WPI consisted out of 89.5% protein, < 0.05% lactose, 0.1% fat, 5% moisture and < 4% ash. The fatty acid composition of the MCT-oil was composed of C 10:0 und C 8:0 fatty acids.

For the interfacial rheological analysis, β-LG was isolated from whey protein isolate (Bipro, Agropur Dairy Cooperative Inc., Minnesota, USA). The method for purification is described elsewhere (Keppler et al., 2014; Schestkowa et al., 2020). The resulting protein had a dry matter content of 90.7 ± 1.0% and a protein content of 90.1 ± 1.2% while the protein content is composed of 98.1% isolated β-LG, 0.4% α-lactalbumin and 1.5% denatured β-LG (analyzed according to Keppler, Sönnichsen, Lorenzen, & Schwarz, 2014). Medium-chain-triglyceride oil (MCT-oil) WITARIX® MCT 60/40 was kindly provided from IOI Oleo GmbH (Hamburg, Germany) and was purified via magnesium silicate adsorption (Florisil®, Carl Roth GmbH, Karlsruhe, Germany) to

remove interfacial active substances. Maltodextrin with a dextrose equivalent of 14 (C*Dry ™ MD 01910) was purchased from Cargill Deutschland GmbH (Krefeld, Germany). The maltodextrin had a protein content of 0.1-0.15% (measured with DUMATHERM, C. Gerhardt GmbH & Co. KG, Königswinter, Germany).

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

2.2 Preparation and spray drying of emulsions

Emulsions were prepared as described in Taboada et al. (2020). Briefly, the emulsions consist of 15 d.m.% (dry matter) MCT-oil with the ratio MCT oil to WPI and LME 1:0.1:0.01 and 24.8 d.m.% (dry matter) maltodextrin. The LMWE were solubilized in oil at 60 °C. An aqueous solution of WPI and the oil containing the LMWE were emulsified for 2 min in a colloid mill (IKA magic LAB®, IKA®-Werke GmbH & Co. KG, Staufen, Germany) operated at a gap width of 0.16 mm and a circumferential speed of 26 m s^{-1}. The maltodextrin was added after the homogenization process. Before spray drying, the emulsions were stored overnight to allow the interfacial film to stabilize.

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

2.3   Storage of spray-dried emulsions

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

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

2.4   Extractable oil of spray-dried emulsions

Extractable oil content was determined gravimetrically with petrol ether as solvent (Westergaard, 2004). Ten grams of powder were solubilized with 50 mL petrol ether in a 100 mL Erlenmeyer flask and mixed for 15 min at 90 rpm on a shaking device. The dispersion was filtered, and 25 mL of the filtrate was transferred in dried and weighed round bottom flasks. The solvent was removed in a rotating evaporator at 65 °C and 700 mbar for 5 min. The evaporated round bottom flasks were weighed after heating for 90 min at 105 °C and cooling in a desiccator. The extractable oil content is provided as percentage of the emulsified oil. This content was measured for each sample at a storage time in duplicate and is shown as mean value with mean deviation.

2.5   Oil droplet size distribution of reconstituted powder

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

2.6   X-ray diffraction of spray-dried emulsions

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

2.7   Colour of spray-dried emulsions

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

2.8   Morphology of spray-dried emulsions

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

2.9   Interfacial shear rheology

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

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

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

3 Results

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

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

Presence of low molecular weight emulsifier also affected the content of extractable oil. In the presence of whey protein and lecithin the extractable oil content amounted to $6.2 \pm 0.2\%$ in comparison to the whey protein stabilized spray-dried emulsion with $7.4 \pm 0.1\%$. In contrast, an increased extractable oil content of $9.5 \pm 0.0\%$ and

10.0 ± 0.0% was observed in spray-dried emulsions stabilized with WPI-mono- and diglyceride or WPI-citrem, respectively (**Figure 2**). These differences in the extractable oil content between powders with different emulsifying constituent combinations remained in a similar order and range during storage. Generally, the extractable oil content remained similar or decreased over time. The only exceptions were powder samples stabilized with WPI-citrem and WPI-mono- and diglyceride based powders stored at -18 °C. In these samples extractable oil increased up to 10.89 ± 0.38% and 10.26 ± 0.18%, respectively. In contrast, lowest extractable oil content induced by temperature stress was observed after 168 days of storage in samples with an initial temperature stress of 60 °C.
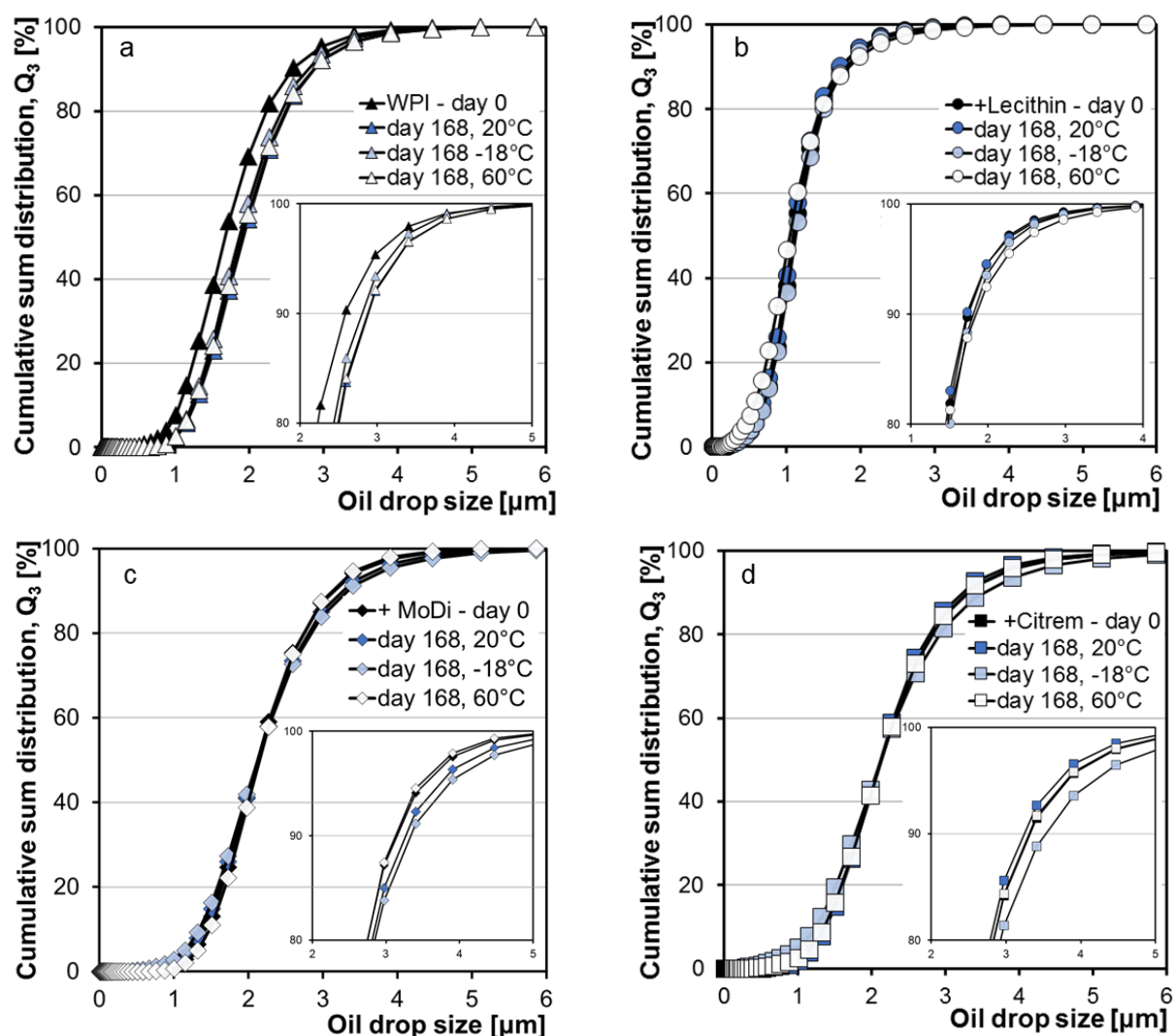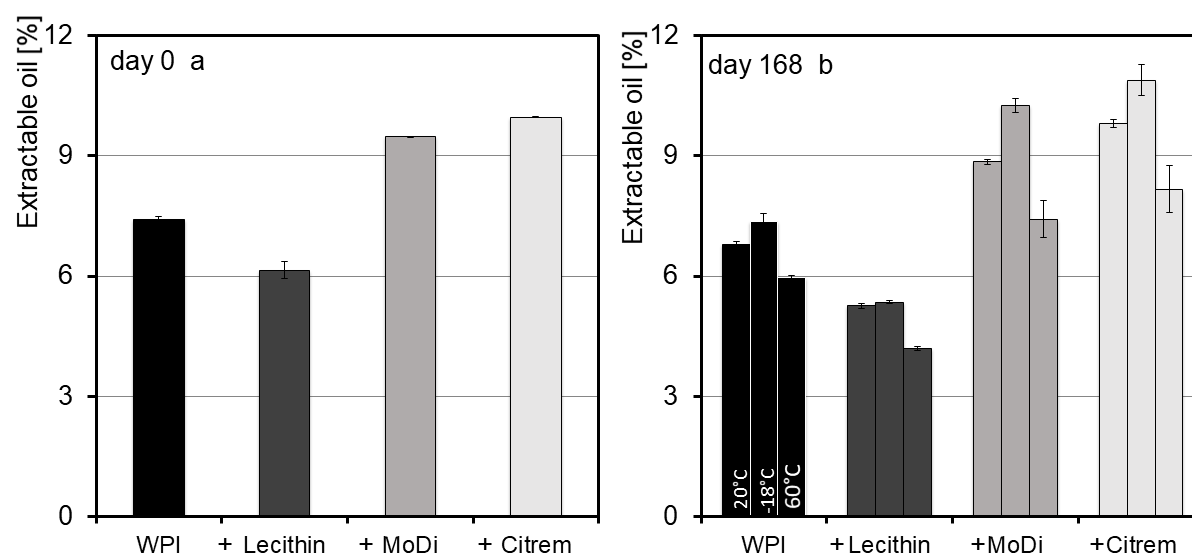

Figure 1: Cumulative sum distribution of oil drop size of spray-dried emulsions which were stabilized by (a) whey protein isolate (WPI) with addition of (b) lecithin, (c) mono- and diglyceride (MoDi) and (d) citrem. The powders were analyzed at day 0 and after storage of 168 days (at 20 °C, -18 °C or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

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

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

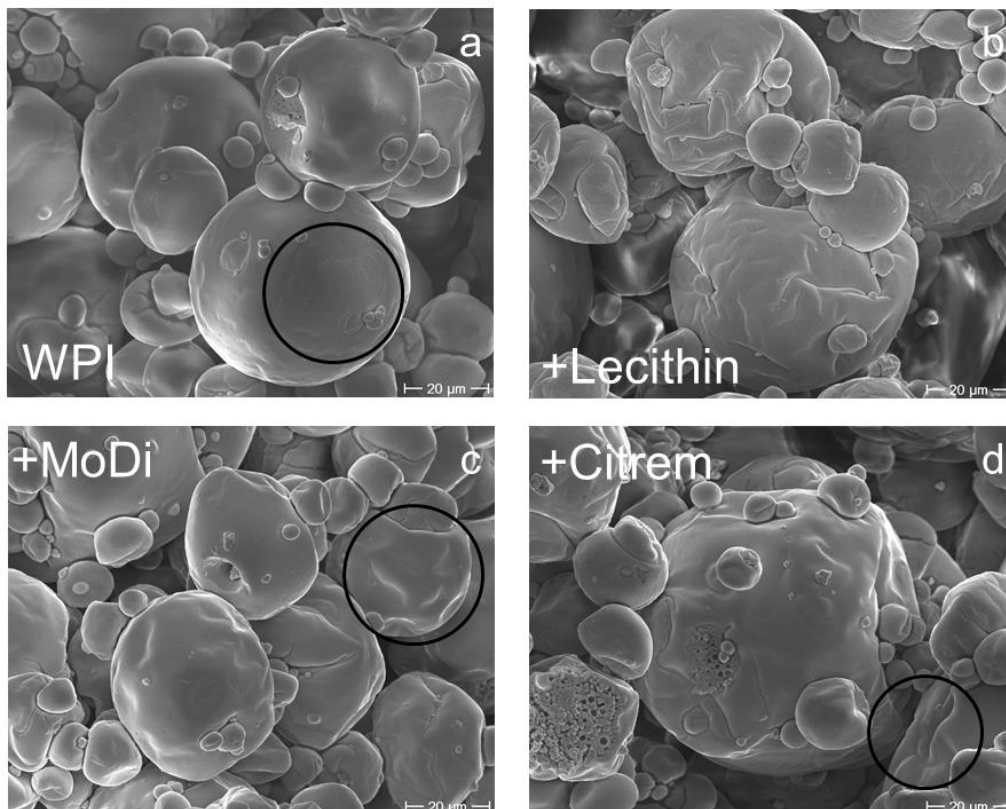[a] Values are presented as mean ± standard deviation of the method.

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

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

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

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

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

Figure 3: Scanning electron microscopy images of spray-dried emulsions at 1000x magnification which were stored at -18 °C and were stabilized by (a) whey protein isolate (WPI) with addition of (b) lecithin, (c) mono- and diglyceride and (d) citrem. Black circles indicate regions with predominantly free fat at surface.
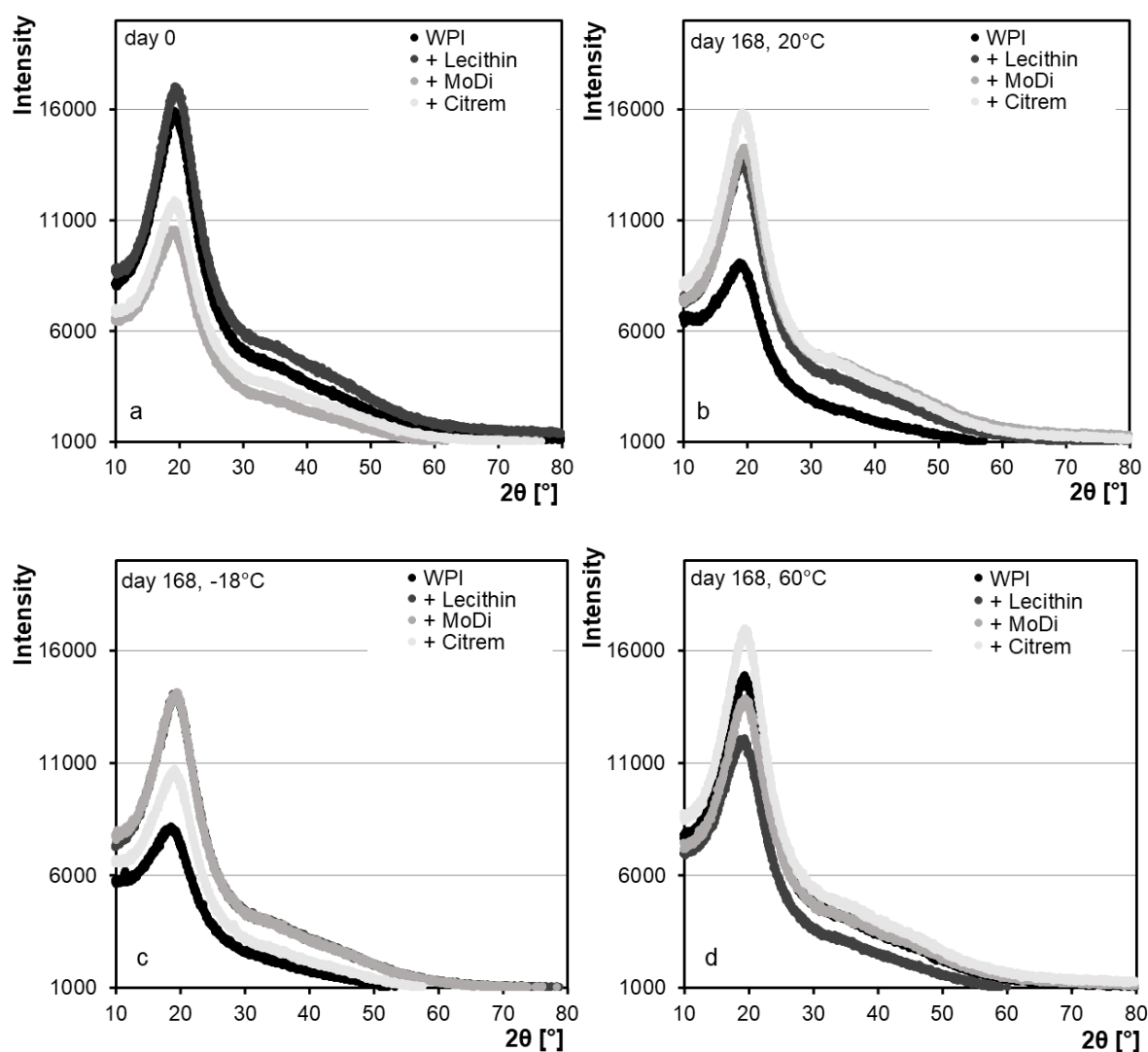
Figure 4: X-ray diffraction (XRPD) patterns of spray-dried emulsions which were stabilized by whey protein isolate (WPI) with addition of lecithin, mono- and diglyceride and citrem. The powders were analyzed at (a) day 0 or after storage for 168 days at (b) 20 °C, (c) -18 °C or (d) 60 °C.

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

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

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

3.3 Interfacial shear rheology

Interfacial shear rheology is applied to analyze the viscoelastic interfacial network and intermolecular interactions at the oil-water interface. **Figure 5** shows the development of the complex shear modulus G* over time for all combinations of emulsifying constituents at the oil-water interface with presence of maltodextrin. The β-lactoglobulin stabilized interface reaches a G* of approximately 30 mN m^{-1} (**Figure 5**). The β-lactoglobulin-lecithin stabilized system showed the lowest G* of 6.4 mN m^{-1} with no change over time. The β-lactoglobulin-citrem and β-lactoglobulin-mono- and diglyceride stabilized interface both showed an initial increase in G* up to 20 and 16 mN m^{-1}, respectively with a slow decrease over time.
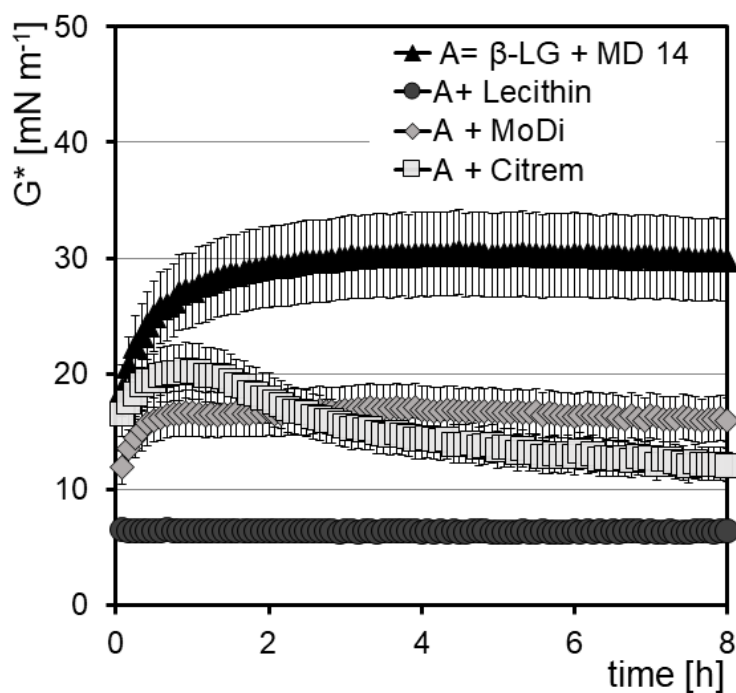
Figure 5: Complex modulus (G*) of 0.1% β-LG film with addition of maltodextrin DE 14 (MD 14) in the aqueous phase and with addition of 0.005% lecithin, mono- and diglyceride and citrem in the oil phase, measured at oil/ water-interface, 1 Hz and 0.001 amplitude. Error bars display the coefficient of variation of the method.

4 Discussion

Selection of the emulsifying constituent already affected the oil droplet size distribution in the feed emulsion. Combination of WPI with LMWE decreased the d_{50} and d_{90} of the oil droplet size distribution compared to WPI alone (**Table 1**). During emulsification, the combinations of emulsifying constituents reduce the oil droplet size of the whey protein stabilized emulsion in dependence on their interfacial activity. It belongs to the well-established knowledge that LMWE have in general a higher interfacial activity than proteins (Murray & Dickinson, 1996) and thus more efficiently stabilize the newly

created droplets during homogenisation. Differences in the interfacial tension of the emulsifier constituent combinations of the present study have already been shown (Taboada et al., 2020). The interfacial tension was lowest for WPI-lecithin followed by WPI-mono- and diglyceride and WPI-citrem (Taboada et al., 2020).

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

In general, in all samples the oil droplet size decreased during spray-drying and particle formation (**Table 1**), which can be attributed to oil droplet break up during atomization (Taboada et al., 2020). It belongs to the well-established knowledge that in this context a viscoelastic interfacial film preserves the stability of spray dried emulsions (Vega & Roos, 2006). Emulsions are typically stored prior to spray drying and this was also the case in the present study. Monitoring the rheological behaviour of the interfacial film over a prolonged period of time is therefore a suitable technique to reveal differences when using combinations of emulsifying constituents. In the present study, the whey protein stabilized interface showed the highest G* indicating that it has the highest viscoelasticity among all samples. It results from strong intermolecular interactions (Murray & Dickinson, 1996; Wilde et al., 2004). Furthermore it is supported by protein enrichment at the interface (Rodríguez Patino & Pilosof, 2011) due to an excluded volume effect as it has been described for β-lactoglobulin and maltodextrin (Heiden-Hecht et al., 2021).

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

In addition, if the newly created interface is not stabilized by the emulsifying constituent, oil droplet coalescence may occur and lead to a shift in the oil droplet size distribution towards an increased oil droplet size. Furthermore, coalescence may occur during water evaporation and particle formation, when oil droplets approach each other due

to a reduction of the volume by evaporation. In this scenario a highly elastic behaviour of the interfacial film offers protection against unintended changes in oil droplet size. It becomes obvious that the properties of the interfacial film of the emulsifying constituents play a key role during the atomization induced break up and potential coalescence. In the present study, the highly interfacial active low molecular weight emulsifier lecithin prevented coalescence and maintained the decrease in oil droplet size. In comparison, in the presence of whey proteins or combinations of WPI and LMWE with a lower interfacial activity like mono- and diglycerides or citrem, oil droplet coalescence occurred to a varying degree and led to a larger oil droplet size as it was earlier described elsewhere (Taboada et al., 2020). A large oil droplet size went hand in hand with a high extractable oil content and vice versa as it becomes obvious when comparing the results in **Table 1** and **Figure 2**a. WPI-lecithin showed the smallest oil droplet size and the lowest extractable oil content followed by WPI, WPI-citrem and WPI-mono- and diglyceride (**Table 1** and **Figure 2**). We assume that after the oil droplet break up during atomization and subsequent coalescence of oil droplets another factor might play a role. Since the time scale from atomization to powder particle formation of spray dried emulsions takes just milliseconds (Taboada et al., 2019; Vega & Roos, 2006), non-stabilized regions of oil droplets may be especially present for WPI, WPI-citrem and WPI-mono- and diglyceride. These non-stabilized regions tend to be not well encapsulated and thus merge with the matrix material. The non-encapsulated oil migrates to the surface or stays in the matrix and can be determined via solvent extraction (Vignolles et al., 2007). A powder with a high extractable oil content is more prone to aggregation and shows a reduced solubility. Therefore, from a practical point of view a low content of extractable oil is a key factor in quality evaluation of spray-dried emulsions.

During storage, changes in oil droplet size distribution and extractable oil content depended on emulsifying constituent combination and temperature stress (**Figure 1** and **Figure 2**). For both, a reduction or an increase of extractable oil content, mechanistic explanations are available. In the present study in WPI-citrem and WPI-mono- and diglyceride stabilized systems exposed to temperature stress at – 18 °C, the extractable oil content and the oil droplet size increased (**Figure 1** and **Figure 2**). In general, crystallization in emulsions requires supercooling, i.e., crystallization temperature is well below the crystallization temperature of the bulk material. Homogeneous nucleation within the oil phase is less likely than heterogeneous nucleation (Garti & Sato, 2001). It is well accepted, that due to the low volume of the oil droplets in an emulsion, volume heterogeneous nucleation due to impurities in the oil is also rare. The major driver for nucleation thus is the so-called surface heterogenous nucleation, where the emulsifier acts as a template for crystallization (McClements, 2012; Ribeiro et al., 2015). When triacylglycerols crystalize, they usually

form α-polymorph, since it is the polymorph with the lowest activation energy, but not necessarily the lowest free energy (McClements, 2012; Ribeiro et al., 2015). As a consequence, polymorphic transitions occur from the α-polymorph through the β'-polymorph to the most stable β-polymorph (McClements, 2012; Ribeiro et al., 2015). Again, polymorphic transitions in an emulsion are much faster than in the bulk material due to a smaller crystal size, and the oil-water interface represents a physical barrier hindering growth (McClements, 2012). In a liquid emulsion polymorphic transition leads to a change in crystal shape from a spherical to a more ellipsoid shape (Awad et al., 2008; McClements, 2012) and crystals may pierce the interface and induce oil droplet aggregation and coalescence (Fredrick et al., 2013; Goibier et al., 2017). Although this deformation may not occur in spray-dried emulsions and mobility of the oil droplets is prevented by their immobilisation in the amorphous matrix, piercing with release of oil may still occur during storage and result in release of encapsulated fat (Fäldt & Bergenståhl, 1995; Millqvist-Fureby, 2003).

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

In contrast, in all samples exposed to temperature stress at 60 °C, the extractable oil content decreased. This decrease in extractable oil may result through formation of protein-fat complexes (Vignolles et al., 2007). The polypeptide chain can interact with the fat in dependence on structural aspects of the protein (Brinkmann et al., 2013). The authors highlight that protein-fat complexes are very likely for an oil phase and whey proteins at 60 °C (Brinkmann et al., 2013; Lišková et al., 2011). This leads to the

conclusion that interactions are hydrophobic in nature and steric effects must also contribute to get a markable effect on extractable oil content. Furthermore, this decrease can be attributed to phase transition phenomena of the matrix material (Roos, 2002; Roos & Karel, 1991; Zafar et al., 2017). For a matrix material of maltodextrin with DE 14 and an a_w-value of 0.35, the glass transition temperature is around 60 °C (Roos & Drusch, 2016). Since the DE is a degree of starch degradation without specific molecular weight profile for the maltodextrins, the differences in molecular weight in the matrix material can induce local phase transition (Hughes et al., 2018). A reduction in extractable oil content due to phase transition of the matrix material could be attributed to the mechanisms of caking. These mechanisms can be, e.g., bridging between particles (Zafar et al., 2017) which can difficult the extractability of the oil in a short and controlled solvent residence time applied in our study.

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

5   Conclusion

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

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