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ORIGINAL RESEARCH PAPER

High-performance thin-layer chromatography for the detection of compositional changes in LACTEM emulsifiers during storage

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Abstract
Quality control of food emulsifiers, such as lactic acid esters of mono- and diacylglycerols (LACTEM), is crucial in the reproducible production of food products. The current study investigated compositional changes of LACTEM emulsifiers using high-performance thin-layer chromatography (HPTLC) during storage at 60 °C for 8 weeks. Ultraviolet (UV) and fluorescence images of the HPTLC silica gel F_{254s} plates after primuline derivatization and densitometric data were analyzed to assess changes in the composition. Significant changes were observed for minor LACTEM components (< 10% relative intensity), specifically a decrease in higher-lactylated monoacylglycerols and an increase in triacylglycerols. Techno-functional properties, such as particle size distribution, apparent viscosity, overrun, foam firmness, drainage, and residual cream of aerosol whipping cream (0.8 g 100 g^{-1} LACTEM) were investigated. While emulsion stability was not affected, the foam firmness increased significantly, corresponding to a visibly more brittle foam. On the basis of these results, monitoring compositional changes in the food-manufacturing process is necessary to maintain constant food quality.

Keywords HPTLC · Food emulsifiers · Quality control · Aerosol whipping cream · LACTEM

1 Introduction

Quality control of food emulsifiers, e.g., lactic acid esters of mono- and diacylglycerols (LACTEM), is essential for ensuring reproducibility in the production of food products containing these emulsifiers. According to European Union (EU) law, they can be used *quantum satis*, as no adverse effects on human health have been reported [1, 2]. Thus, LACTEM emulsifiers are applied in various food products to adjust techno-functional properties, e.g., in aerosol whipping cream.

Structurally related emulsifiers include monoacylglycerols (MG) and mono- and diacylglycerols (MG/DG), commonly referred to as E 471 in the EU. Blankart et al. demonstrated that the composition of these emulsifiers changes during storage at temperatures above their melting point [3]. Over an 8-week storage period, they observed a decrease in MG, while levels in DG increased. Furthermore, the rearrangement of 1,3- into 1,2-DG was noted. While the rearrangement of DG did not lead to changes in techno-functional properties, the concurrent decrease in MG and increase in DG led to increased drainage. The effect on techno-functional properties varied depending on the composition of the emulsifier, such as the degree of unsaturation and the MG/DG ratio [3].

Similarly, LACTEM emulsifiers may also be prone to temperature-induced changes during storage. This is particularly relevant as LACTEM emulsifiers, such as MG and MG/DG, can be exposed to temperature fluctuations during transportation, storage, and processing. In his compendium on food emulsifiers, Norn described storage of LACTEM emulsifiers at 60 °C for up to 2 weeks that resulted in an increased acid value, which was attributed to the hydrolysis of lactic acid esters. This hydrolysis was more pronounced in higher-lactylated emulsifiers (18–20% lactic acid). Furthermore, a decrease in MG was also observed over storage periods of 12 days at 60 °C and 90 °C [4]. Despite these findings, studies exploring

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the impact of such compositional changes on the techno-functional properties of LACTEM emulsifiers in food products remain scarce. However, this is a critical issue for food manufacturers as ensuring a reproducible production process can prevent recalls that can lead to economic losses and reputation damage.

High-performance thin-layer chromatography (HPTLC) was shown to be an efficient tool for analyzing LACTEM emulsifiers. In previous studies conducted by our working group, characteristic signals of the HPTLC fingerprint of LACTEM emulsifiers separated on silica gel and derivatized with primuline were identified and assigned to lactylated MG and DG with varying degrees of lactylation [5, 6]. HPTLC has also been recognized in literature as being well-suited for quality control, such as control of herbal drugs [7, 8] and food manufacturing processes [9, 10].

Thus, the study aimed to investigate compositional changes of LACTEM during storage at temperatures above the melting point (60 °C) and assess their impact on techno-functional properties, e.g., mean particle size, apparent viscosity, overrun, foam firmness, and drainage, in aerosol whipping cream. HPTLC was selected as the method of choice owing to its easy implementation and successful establishment in our laboratory for the analysis of LACTEM emulsifiers, as reported in previous publications [5, 6].

2 Experimental

2.1 Chemicals

Chloroform (Chromasolv, for residue analysis, ≥ 99.9%) was provided by Sigma-Aldrich (Steinheim, Germany). Diethyl ether (≥ 99.5%) was obtained from Thermo Fisher Scientific (Schwerte, Germany). *n*-Heptane (Chemsolute, for HPLC, ≥ 99.2%), methanol (Chemsolute, LC−MS, > 99.9%), and methyl *tert*-butyl ether (MTBE, Chemsolute, for HPLC, ≥ 99.8%) were purchased from Th. Geyer (Renningen, Germany). Ultrapure water (> 18 MΩ cm) was supplied by a Millipore Synergy System (Schwalbach, Germany). Formic acid (analytical reagent grade, > 98%) was provided by Thermo Fisher Scientific. Magnesium chloride was obtained from Merck (Darmstadt, Germany), and potassium carbonate (K_2CO_3, anhydrous, for analysis, ≥ 99%) was purchased from Carl Roth (Karlsruhe, Germany). Primuline (dye content 50%), stearic acid (> 99.5%), 1-stearoyl-*rac*-glycerol (> 99%), 1,2-distearoyl-*rac*-glycerol (> 99%), 1,3-distearoylglycerol (> 99%), and glyceryl tristearate (> 99%) were provided by Sigma-Aldrich. HPTLC silica gel F_{254s} MS-grade plates from Merck were used without pre-washing. A technical grade LACTEM emulsifier sample was provided by a manufacturer.

2.2 Sample and standard solutions

Sample solutions of the stored emulsifier samples were prepared at a concentration of 500 mg L^{-1} in MTBE. Between measurements, sample solutions were kept in the refrigerator at 4 °C. Stock solutions of stearic acid (SA), 1-monostearate (1-MSt), 1,2- and 1,3-distearate (1,2-DSt and 1,3-DSt), and tristearate (TSt) were prepared in MTBE at 1 mg mL^{-1}. A standard mix solution was prepared at 0.2 mg lipid class mL^{-1} and used for comparison. For the lactylated acyglycerols, no analytical standards are available. Identification relies on a previous study [5].

2.3 Storage of a LACTEM emulsifier

The LACTEM emulsifier was stored at 60 °C in a closed Schott flask in a heating cabinet (T5042, Heraeus, Hanau, Germany). Because the LACTEM was in liquid state at this temperature, 60 °C was chosen. After distinct storage times (0 h, 1 h, 2 h, 4 h, 8 h, 24 h, 168 h (1 week), 336 h (2 weeks), 672 h (4 weeks), and 1344 h (8 weeks)) samples were collected and stored in the refrigerator prior to HPTLC analysis (Sect. 2.6). For the aerosol whipping cream manufacturing (Sect. 2.4), the same samples were used, except for the 0-h storage point, where values determined in preliminary experiments for the same emulsifier were applied.

2.4 Manufacturing of aerosol whipping cream

The aerosol whipping cream was manufactured according to [6]. In short, 0.8 g 100 g^{-1} emulsifier was added to pasteurized cream (lipid content of 30 g 100 g^{-1}). This emulsifier content was found to be the saturation content in preliminary experiments. After pre-emulsification, the samples were homogenized with a two-stage homogenizer (6/1 MPa). Then, the samples were collected in flasks, cooled in ice water, and stored at 4 °C for 24 h in a plate heat exchanger. After that, rheological properties were conducted. Foaming was performed according to [11] with nitrous oxide (15 g in total).

2.5 Determination of techno-functional properties

2.5.1 Particle size distribution

Particle size distribution in aerosol whipping cream was performed as described in previous publications, using static light scattering with a LS 13320 (Beckmann-Coulter, Brea, CA, USA) and a sample injection volume of 100 µL [3, 11]. For protein-stabilized fat globules, a refractive index of 1.46, as reported by [12], was applied. Characterization was based

on the $D_{90,3}$, the diameter that 90% of the particles do not exceed in volume-based evaluation. Each sample was analyzed three times in triplicate or quintuple.

2.5.2 Rheological properties

Rheological measurements of liquid samples were conducted on a MCR 502 and a MCR 302 (Anton-Paar GmbH, Graz, Austria) equipped with a coaxial cylindrical geometry ($d_o = 27$ mm, $d_i = 25$ mm, $l = 40$ mm) with a measuring gap of 1 mm. In total, 13 g of the sample was weighed into the geometry and equilibrated for 10 min at 5 °C. The shear rate profile was as follows: from 0 s^{-1} to 500 s^{-1} over 250 s, kept at 500 s^{-1} for 250 s, and decreased from 500 s^{-1} to 0 s^{-1} over 250 s. Apparent viscosity was calculated when the shear rate reached 500 s^{-1}. Samples were analyzed two times in triplicate or quintuple.

2.5.3 Overrun

Overrun was calculated by differential weighing according to [11]. The density of the model aerosol whipping cream was determined to 1010 kg m^{-3} at 5 °C using a DMA 5000 (Anton-Paar GmbH, Graz, Austria). Samples were analyzed two times in triplicate.

2.5.4 Foam firmness

Foam Firmness was analyzed according to [3] with a universal testing machine (5944; Instron, Norwood, USA) using a crosshair probe (0.1 cm wire diameter). Foam firmness was defined as the arithmetic mean of the last 20 measurement points. Samples were analyzed two times in triplicate.

2.5.5 Drainage

Drainage was assessed according to [3]. The amount of drained serum was determined by differential weighing and normalized to the amount of foam used. Samples were analyzed two times in triplicate.

2.5.6 Residual cream

After overrun measurements and discarding of the remaining sample, residual cream was calculated according to [3] by differential weighing. Samples were analyzed two times in triplicate.

2.6 High-performance thin-layer chromatography–fluorescence detection

HPTLC coupled to fluorescence detection (FLD) were carried out as described by [6]. Briefly, 10 µL of the sample solution (resulting in 5 µg LACTEM per zone) were applied bandwise (band length 5 mm) on 20 cm × 10 cm plates with an Automatic TLC Sampler 4 (CAMAG, Muttenz, Switzerland). The distance from the lower edge was 8 mm and from the left and right edge, 10 mm. MTBE was used as the rinsing solvent with one rinsing and filling cycle. Following application, the plates were dried in a fume hood for 10 min. Subsequent twofold development was performed in an Automatic Developing Chamber (ADC2, CAMAG) equipped with a 20 cm × 10 cm twin-through chamber (CAMAG). Before each development, plate activity was set by the automatic humidity control unit of the ADC2 using a saturated magnesium chloride solution (relative humidity of 33%). The first development was carried out using a mixture of chloroform–methanol–water–formic acid (67:6:1.2:0.2, *V/V*) up to a migration distance of 50 mm, followed by a 10 min drying step. The two-phase solvent system was well shaken until being filled in the developing chamber briefly before the start of the development. For the second development, a mixture of *n*-heptane–diethyl ether–formic acid (55:45:1, *V/V*) up to a migration distance of 80 mm was applied, including 5 min of drying. After every working step, plate images were captured with the TLC Visualizer (CAMAG) under UV 254 nm and UV 366 nm illumination. For visualization of the LACTEM components, plates were dipped into a solution of primuline (0.05% in acetonewater, 4:1, *V/V*) with the TLC Chromatogram Immersion Device III (CAMAG, immersion speed: 1, immersion time: 2) and dried in a stream of cold air for 1 min. The plates were then stored in a desiccator at a constant relative humidity of 47% adjusted by a saturated K_2CO_3 solution for 1 h. After that, plate images were captured once more. Densitograms were obtained by scanning the plates in fluorescent mode at UV 366 nm with the TLC Scanner (CAMAG) using the mercury lamp with the optical filter being set to K400 and an analog offset of 10%. Control of the HPTLC instruments and data evaluation was performed with the software winCATS, version 1.4.6.2002 (CAMAG).

2.7 Statistical analysis

Seven characteristic LACTEM emulsifier signals were selected, and their intensities at each storage point were determined by HPTLC–FLD ($n = 5$). To detect significant changes in signal intensities over time and identify where significant changes occur, one-way analyses of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test were performed at a 95% significance level. Measurement uncertainty was calculated as margin of error at the same significance level. Statistical analyses were conducted using RStudio, version 2024.09.0 (Posit Software, PBC, Boston, MA, USA), running on R, version 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Plots were created using OriginPro, version 10.1.0.178 (OriginLab Corporation, Northampton, MA, USA).

3 Results and discussion

3.1 Changes in the HPTLC fingerprint of stored LACTEM

The chromatographic fingerprint resembled those of typical LACTEM emulsifiers described by [6], showing seven signals (Fig. 1, S1–S7). As the same analytical conditions were used, the signals were assigned to MG and lactic acid esters of MG (S1–S4), DG and lactic acid esters of DG (S5–S6), and TG (S7), as reported by [6]. Within the signal group of the MG and lactic acid esters of MG (S1–S4), the degree of esterification by lactic acid increases with increasing hR_F value. Most signals were present throughout the entire storage period (S1–S3, S5–6), while some signals appeared (S7) or disappeared (S4) with increasing storage time. Notably, S7 was detected more sensitively by densitometry, emphasizing the higher sensitivity of the TLC scanner compared with the TLC visualizer. It was hypothesized that with increasing storage time, the content of higher-esterified compounds and, consequently, their signal intensity, would decrease. This can be observed on the plate image (Fig. 1, S4 and S3). While S4 is clearly visible up to a storage time of 8 h, it diminished completely at 672 h (4 weeks). Similarly, the S3 intensity decreases, though it remains visible throughout the entire storage period. Surprisingly, the intensity of S7, representing TG, increases over time. Since the data are reported as percentages, a closure effect cannot be ruled out. However, the clear appearance of the TG signal at longer storage times suggests an actual formation rather than a mere relative increase. Nevertheless, it has to be noted that primuline is a nonspecific derivatization agent that also reacts with other compounds having a long alkyl chain [13]. Identification by coupling with mass spectrometry might lead to clarification.

A more detailed insight into the compositional changes of the LACTEM emulsifier was obtained through densitometry (Fig. 2). The calculated relative signal intensities over the storage period showed a significant decrease in S3 (from 9.4% to 5.9% after 672 h (4 weeks)) and S4 (from 1.8% to not detected after 336 h (2 weeks)), supporting the hypothesis that higher-lactylated LACTEM components are more susceptible to hydrolysis. While a decrease in the signal intensity for S2 was also detected starting at 168 h (1 week), this decrease was not statistically significant. In contrast to the storage of E 471 emulsifiers, no reduction in MG was observed [3]. This discrepancy is attributed to the hydrolysis of lactylated compounds, leading to the formation of MG. A chemical equilibrium might exist between the formation and hydrolysis of MG.

Blankart et al. also described either the formation of DG for a saturated MG emulsifier or the rearrangement of 1,3-DG to 1,2-DG for a saturated MG/DG emulsifier upon storage of different E 471 at temperatures above the melting point of the specific emulsifier [3]. In the current study, only a slight increase, although not statistically significant, was observed for S5 and S6 (DG and lactic acid esters of DG).

Free fatty acids (FFA) or an increase in FFA could not be detected at any storage point, which aligns with the findings of [3], who detected FFA but no increase over the storage period. With the current method, detection of lactic acid was not possible. Determination of free lactic acid by high-performance liquid chromatography coupled with UV detection, according to [14], showed a significant increase in free lactic

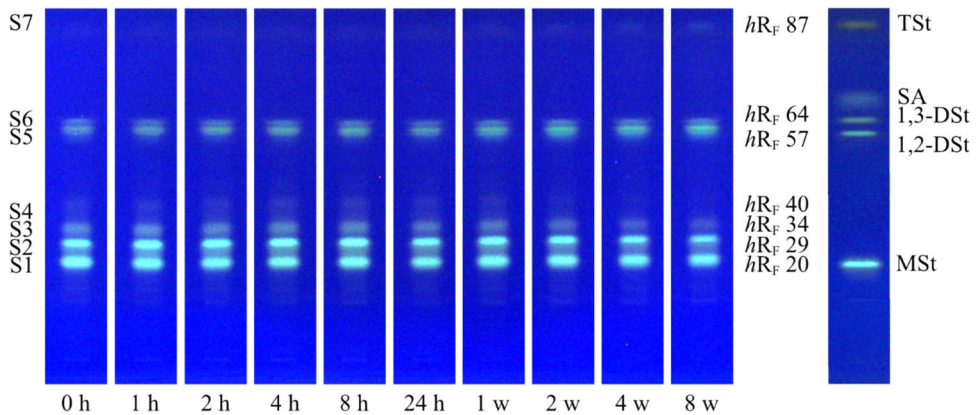

Fig. 1 HPTLC separation of a LACTEM emulsifier (5 µg per zone) stored at 60 °C, sampled at different time points (0 h to 8 weeks (w) storage), fluorescence detection after UV 366 nm excitation following impregnation with primuline. Separation was performed on HPTLC silica gel F_{254s} MS-grade plates with a twofold development (chloroform–methanol–water–formic acid (67:6:1.2:0.2, *V/V*) up to 50 mm, and *n*-heptane–diethyl ether–formic acid (55:45:1, *V/V*)) up to 80 mm. For comparison, a standard mix solution of 1–monostearate (MSt), 1,2- and 1,3-distearate (DSt), stearic acid (SA), and tristearate (TSt) was applied (each 1 µg per zone)

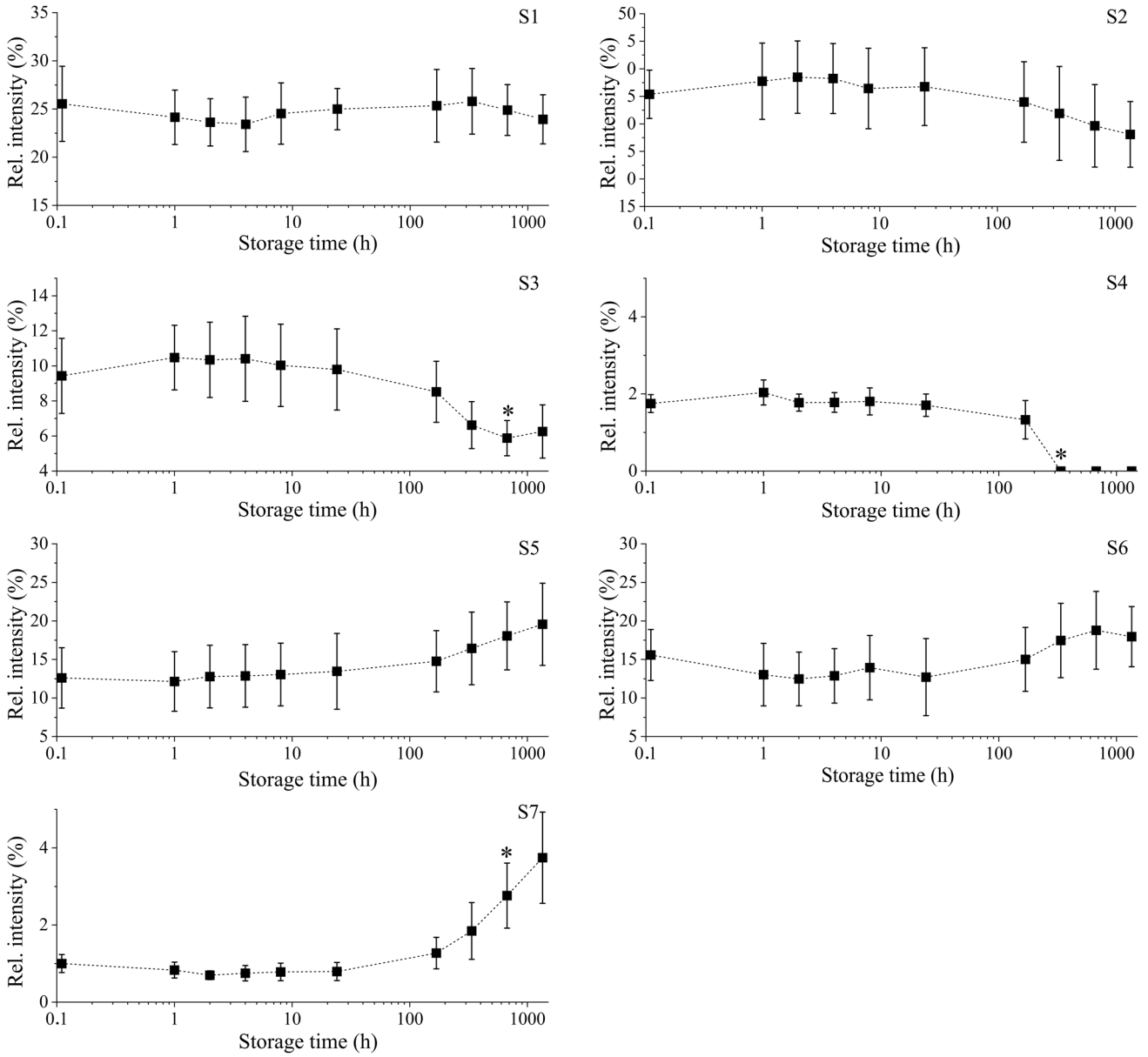

Fig. 2 Relative intensity (%) of the selected signals (S1–S7) of a LACTEM emulsifier in the densitogram obtained at UV 366 nm with an K400 optical filter, plotted against the storage time (h) of the emulsifier stored at 60 °C. For the logarithmic representation, storage time = 0 h was replaced by the pseudo value time = 0.1 h. Measurement uncertainty, calculated as the margin of error, is represented by the error bars. Significant differences to 0 h storage time are indicated by an * (ANOVA, Tukeys-HSD, $\alpha = 0.05$)

acid starting at a storage point of 168 h (1 week, $\alpha = 0.05$, data not shown). This finding is in accordance with previous studies described by [4]. An increase in free lactic acid might lead to a decrease in pH, which impacts the protein structure and has already been shown to affect the foaming properties of skim milk powders [15].

In contrast to the study by [3], a significant increase in the signal intensity of TG was detected after a storage period of 1 week (168 h). Still, the relative intensity remained low, at $3.7 \pm 1.2\%$.

The current study showed that significant compositional changes in LACTEM components occurred upon storage. Those significant changes were primarily observed for minor components (< 10% relative intensity), such as S4 and S7. In addition, the compositional changes of LACTEM emulsifiers seem to differ from E 471 emulsifiers [3], as discussed above, despite their structural similarity.

3.2 Changes in the techno-functional properties of aerosol whipping cream

To evaluate whether the changes observed in the HPTLC fingerprint were reflected in the techno-functional properties, the Department of Soft Matter and Dairy Science of the University of Hohenheim conducted tests regarding emulsion and foam stability in aerosol whipping cream.

No trends were observed for the particle size and the apparent viscosity, indicating that compositional changes of the LACTEM emulsifier, namely the decrease of higher-lactylated compounds and the increase in TG, did not negatively impact emulsion properties (Table 1). Furthermore, no effects were noted for overrun and residual cream (Table 1). These findings are consistent with results reported for E 471 emulsifiers [3].

The drainage showed a u-shaped trend. It decreased significantly until a storage time of 168 h (1 week) and, after, increased again to its initial value at 1 h. In contrast, Blankart et al. reported a significant increase in the drainage of a saturated MG emulsifier [3]. This has also been noted for a decrease in MG and an increase in DG content before [11].

With prolonged storage time of the LACTEM emulsifier, the foam firmness increased significantly (Table 1). However, this did not indicate improved foam stability, as the foam was getting more brittle and even showing cracks at the surface. Significant changes were only observed using the 1-h storage time as a reference. The value for the storage time of 0 h was significantly higher than that of 1 h. A significant deviation from 0 to 1 h storage time was also observed for the drainage. A reason could be that the values for the reference at 0 h were conducted considerably earlier, with different raw material and a different operator. This highlights the high complexity of foam analysis, even when the cream's raw material (protein and fat content) and the operating procedure are standardized. Thus, for the design of future studies, it is important to measure the reference point in the same batch as the storage points. When a saturated MG emulsifier was investigated, Blankart et al. observed a decreased foam firmness over the storage time, which they attributed to the reduction of the MG content, as reported in their previous study [3]. In contrast, for the tested saturated MG/DG emulsifier, the authors did not observe any influence of the storage time [3, 11].

4 Conclusions

The current study used HPTLC to detect storage-induced compositional changes of LACTEM emulsifiers stored at 60 °C over 8 weeks and related those changes to the techno-functional properties of aerosol whipping cream. Over time, higher-lactylated MG decreased while TG increased. Overall, the compositional changes mainly affected minor constituents (< 10% relative intensity). The foam firmness was identified as the most sensitive among the tested techno-functional properties. In contrast, storage did not affect emulsion properties, indicating that the compositional changes mainly affect foaming properties.

The results presented in this study and previous research also highlight that the effects of storage vary strongly among structurally related emulsifiers. Thus, food manufacturers need to know the exact composition of the applied emulsifier to ensure a reproducible production process and prevent recalls, which can lead to economic losses and reputation damage. Those compositional changes affecting the techno-functional properties might also occur at lower temperatures

Table 1 $D_{90,3}$ (µm), apparent viscosity (mPa s), foam firmness (mN), overrun (%), and normalized drainage (%) of aerosol whipping cream (0.8 g 100 g^{-1} LACTEM emulsifier, 30 g 100 g^{-1} fat) in dependence of the storage time at 60 °C

Storage time (h)	$D_{90,3}$ (µm)	Apparent viscosity (mPa s)[a]	Foam firmness (mN)	Overrun (%)	Normalized drainage (%)
0	1.9 ± 0.2	53 ± 10	358 ± 19.0	500 ± 16.7	19 ± 6.2
1	1.6 ± 0.2	49 ± 21	252 ± 36.1	498 ± 23.4	49 ± 18
2	1.9 ± 0.7	61 ± 19	274 ± 49.8	478 ± 25.1	52 ± 13
4	1.8 ± 0.1	51 ± 8.3	276 ± 42.8	495 ± 26.1	37 ± 4.2
8	1.7 ± 0.3	47 ± 4.1	262 ± 58.7	497 ± 32.1	42 ± 11
24	1.8 ± 0.1	47 ± 6.4	319 ± 35.8	475 ± 39.8	34 ± 11
168	1.9 ± 0.4	56 ± 3.3	343 ± 16.2*	464 ± 20.0	20 ± 11
336	2.4 ± 1.1	52 ± 9.5	406 ± 64.0*	475 ± 21.2	42 ± 13
672	3.0 ± 0.2	52 ± 9.8	339 ± 85.6*	458 ± 24.3	45 ± 18
1344	1.9 ± 0.1	57 ± 8.2	395 ± 21.5*	481 ± 25.6	44 ± 6.0

Values are shown as arithmetic means ± measurement uncertainty, calculated as the margin of error ($\alpha = 0.05$). Except for the storage time of 0 h ($n = 3$), independent repetitions were performed two times ($n = 2$)

[a]At $\dot{\gamma} = 500\ s^{-1}$ at 5 °C, * significant difference to storage time 1 h (ANOVA, Tukey-HSD, $\alpha = 0.05$)

but slower. This should be investigated in further studies to assess their relevance to the industry. The presented HPTLC method has proven to be strongly reliable, with consistent results, and detecting even minor compositional changes.

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Data availability All recent data are available from the corresponding author upon reasonable request.

Declarations

Conflicts of interest The last author, Claudia Oellig, is a member of the Editorial Board of the journal. Therefore, the submission was handled by a different member of the editorial board, and she did not take part in the review process in any capacity. The authors have no relevant financial or nonfinancial interests to disclose.

Research involving human and animal participants This article does not contain any studies with human or animal subjects.

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