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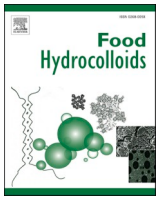

Coalescence of oil droplets stabilised with β-lactoglobulin and/or phospholipids. A microfluidic study dedicated to the impact of temperature and phospholipid type

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ABSTRACT

In scientific emulsification work, the focus is often on a single component and how it behaves at time scales that are at least seconds but often much longer. In practice, emulsions would be produced using mixtures, with droplet formation times in the millisecond range and below. In the current paper, we use a relevant combination of components, β-lactoglobulin (β-LG) and various phospholipids (PL), and investigate them through a microfluidic device that can be used to monitor droplet stability at sub-second time scales. In contrast to conventional emulsion methods, which only permit post hoc analysis, the microfluidic setup provides direct insight into the emulsification process immediately after droplet formation, enabling evaluation of the interplay between interfacial adsorption and droplet coalescence. Droplet stability was greatly enhanced when using a combination of β-lactoglobulin and phospholipids, compared to droplets stabilised by one of the components. For example, for β-LG + 0.005 wt% PL stabilised emulsions the extent of coalescence remained low (number of coalescence events $N_{coal} < 1$) for adsorption times of 31 ms for β-LG concentrations of 0.001 wt% or higher. In comparison, purely β-LG stabilised emulsions were unstable under the same conditions, even at the highest β-LG concentration tested (0.01 wt% β-LG, $N_{coal} = 2.4$) as was the case for the purely PL stabilised emulsions ($N_{coal} \gg 1$). There is clearly a synergistic effect that leads to enhanced droplet stability against coalescence. This was even more pronounced at high temperatures. For instance, at 50 °C β-LG + PC 18:1 emulsions were stable, even at β-LG concentration as low as 0.0001 wt% ($N_{coal} < 1$). At elevated temperatures, only minor effects on coalescence stability related to the PL's molecular structure were observed. The insights obtained with microfluidics differed from those obtained for emulsions, where displacement was noted amongst other factors. Apparently, the time scales used in the current work were too short for this to occur, as this is expected to be a phenomenon occurring over at least minutes. From the experiments carried out here, clues can be derived for formulating stable emulsions and choosing suitable production processes. Amongst others, the protein and phospholipid should be carefully chosen, depending on the temperatures used during/after emulsification.

1. Introduction

During production and/or further processing of oil-water emulsions, the droplets are mechanically and thermally stressed, which can cause aggregation, coalescence, and, eventually, phase separation. The choice of emulsifier is crucial as it influences the interfacial properties and, thus, the coalescence susceptibility of the droplets.

Commercial oil-water emulsions often contain a mixture of surface-active ingredients, leading to rather complex interfacial behaviour (McClements & Jafari, 2018). In dairy products, for instance, phospholipids (PL) are naturally present and contribute (together with the dairy proteins) to stabilising the emulsion. This is because protein-PL interactions lead to a higher number of protein-protein crosslinks on the interface, consequently increasing the interfacial elasticity (Dickinson & Yamamoto, 1996; He et al., 2008). However, in some cases the addition of PLs leads to the destabilisation of the emulsion due to the

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displacement of adsorbed proteins (Bos & van Vliet, 2001; Gomes et al., 2018; Grigoriev et al., 2007; Nylander et al., 2019a), which has been related to the higher decrease in Gibbs interfacial energy of the PL (Bos & van Vliet, 2001; Nylander et al., 2019b). Therefore, when working with mixtures, it is not only important to characterise their individual interfacial properties but also when present in a mixture. In addition, using PL in conjunction with proteins comes with challenges during preparation. The PL usually requires a pre-heating step to dissolve fully and to cause a phase transition from a solid to a liquid state, which implies that the emulsification step needs to be carried out at temperatures above the PL's melting point (>50 °C). This, in turn, may affect the protein structure; e.g., β-lactoglobulin (β-LG) denatures above 70 °C (Loveday, 2016).

In previous studies, we have shown that the PL's molecular structure (head group, fatty acid chain) impacts the interfacial packing and resulting interfacial properties (Risse, Bridot, Yang, et al., 2025b), also when used in combination with proteins (Risse, Bridot, Bäther, et al., 2025a). The PL's fatty acid chain determines whether interactions between PL and β-LG occur, while the head group dictates the strength of interactions (Risse, Bridot, Yang, et al., 2025b). While these results obtained using model interfaces (i.e., interfacial dilatational and shear rheology) help to predict the storage stability of an emulsion, they do not relate to the actual emulsification step where interfaces are formed with milliseconds, and emulsifier adsorption is driven by convective mass transport rather than diffusion (Schroën et al., 2020). To quantify droplet stability at this short time scale (milliseconds to second range) and under mild flow conditions, a microfluidic coalescence cell can be used (E. B. A. Hinderink et al., 2020b, 2021; Krebs et al., 2012a, 2013; Muijlwijk et al., 2017; Muijlwijk, Hinderink, et al., 2016; Soc et al., 1988; Wang et al., 2020).

The adsorption time can be varied in these microfluidic systems (usually from 11 to 173 ms) to test how fast the emulsifiers stabilise the oil droplets (Muijlwijk et al., 2017), and the coalescence rate within the cell can be calculated based on image analysis. Adsorption times are similar to the residence time encountered in the dispersing zone for rotor-stator or high-pressure emulsification systems (Jafari et al., 2008). Therefore, the insights obtained through microfluidics are expected to be helpful in adjusting emulsion formulation and processing conditions to achieve stable emulsions. Until now, this setup has been used for different surfactants such as Tween 20 or sodium dodecyl sulphate (Bera et al., 2021; Brigodiot et al., 2024; Krebs et al., 2012b), particles (Schröder et al., 2018) and different proteins (E. B. A. Hinderink et al., 2020a; Muijlwijk et al., 2017; R. Zhang et al., 2024).

Only very little work was done on the combination of surfactants (Muijlwijk, Huang, et al., 2016); most of the work was dedicated to droplets stabilised by either protein or surfactant, not to their combined behaviour. To the best of our knowledge, such short-timescale measurements have not previously been applied to mixed protein-PL systems. Understanding the interfacial behaviour of such mixed systems at very short timescales is essential, as proteins and PL often compete for interfacial adsorption, and the initial adsorption directly influence droplet stability in emulsification processes. Furthermore, the role of temperature during emulsifier adsorption has so far received limited attention, although PLs often require elevated processing temperatures due to their low solubility at room temperature. To date, only few microfluidic studies have been conducted at elevated temperatures. For instance, for SDS and Tween 20 stabilised droplets, the coalescence frequency increased with increasing temperature due to the reduced viscosity of the liquid phases, which may lead to stronger perturbations in the thin film separating the droplets (Bera et al., 2021). Performing microfluidic experiments at elevated temperatures for protein-PL mixtures is thus a crucial next step in understanding their behaviour during emulsification and to rational design these processes and represents an additional novel aspect of this study.

In the current paper, we combine proteins with different PLs and study the resulting coalescence stability after various adsorption times (31–173 ms). We distinguish effects related to the PL's molecular structure by varying the headgroup and the fatty acyl chain. We study this not only at room temperature but also at typical temperatures used in industrial processes (20 and 90 °C) to investigate the effect of protein denaturation and the PL's phase transition (which can be very different depending on the PL type). We selected phosphatidylcholine (PC) and phosphatidylethanolamine (PE) based on their natural origin and relevance in food- and bio-based emulsifier systems. These PLs are among the main components of commercial lecithin products and are widely used in the formulation of emulsions in the food, pharmaceutical, and cosmetic industries. Importantly, both PC and PE are available in saturated (hydrogenated) and naturally unsaturated forms, which allows for a systematic investigation of the impact of FA saturation on interfacial behaviour and protein-PL interactions.

2. Material and methods

2.1. Materials

The PLs used were 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine 18:0 PE (DSPE) (CAS 1069-79-0) from Sigma Aldrich (St. Louis, United States), 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphoethanolamine 18:1 (Δ9-Cis) PE (DOPE) (4004-05-01), 1,2-dioctadecanoyl-sn-glycero-3-phosphocholine 18:0 PC (DSPC) (CAS 816-94-4) and 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine 18:1 (Δ9-Cis) PC (DOPC) (CAS 4235-95-4) from Avanti Polar Lipids Inc (Alabaster, United States).

Medium chain triglyceride oil (MCT-oil) WITARIX® MCT 60/40 was kindly provided by IOI Oleo GmbH (Hamburg, Germany).

Whey protein isolate was kindly provided by Formo Bio GmbH (Berlin, Germany). The β-lactoglobulin was isolated according to (Keppler et al., 2014; Kieserling et al., 2021). The obtained β-LG isolate had a protein content of 94.26 wt%, of which 97 wt% was β-LG.

The MCT oil consisted of triglycerides with fatty acid chains between C6:0 and C12:0 (56.4 wt% C 8:0 and 43.3 wt% C 10:0 triglycerides). The MCT oil was purified with magnesium silicate (Florisil® from Carl Roth GmbH, Karlsruhe, Germany) for 24 h to remove interfacial active substances.

2.2. Sample preparation

2.2.1. Preparation of the β-LG solutions

β-LG solutions with varying protein concentrations of 0.0001 wt% to 0.01 wt% were prepared in Milli Q water, the pH was adjusted to pH 6.50 using 0.1 M HCl, and the solution was stirred overnight at 20 °C to fully hydrate the protein. The pH of the solutions was adjusted to pH 6.50 using 0.01 M HCl or 0.01 M NaOH the next day if necessary. Before use, the β-LG solutions were filtered (1 μm PES filter, Merck KGaA, Darmstadt, Germany).

2.2.2. Preparation of the PL solutions

The four PLs (PE 18:0, PC 18:0, PE 18:1, PC 18:1) were dissolved in MCT oil at 0.005 wt% and heated to temperatures above the melting point, i.e., 130 °C (saturated) or 110 °C (unsaturated PL) to completely dissolve the phospholipids (Risse, Bridot, Bäther, et al., 2025a). The temperature was held for 30 min to exclude fat memory effects. Afterwards, the PL-solutions were cooled down to room temperature. During this step, no optical change was observed, i.e., there was no sign of PL crystallisation. Although no optical changes were observed, the PL oil solutions were filtered (1 μm PES filter, Merck, Germany) before use to avoid blockage of the chips due to dust.

The PL oil solution was used either directly after preparation (measurements at T 20 °C) or reheated to the desired temperature of the microfluidic experiment (measurements at higher temperatures).

2.3. Contact angle measurements of the PL – solutions on glass

For all four PL solutions, contact angle measurements were carried out by sessile drop tensiometry (Tracker, Teclis Scientific, Civrieux-d'Azergues, France) to test the PL's wettability behaviour on a glass wall surrounded by an aqueous phase (model system for chip) (supplementary material, figure S-1). This information was needed to predict if it would be possible to create PL-oil droplets in the microfluidic setup or if the PL-oil solution would start "creeping" along the oil channel. Therefore, a cuvette was filled with distilled water, and an oil droplet of a defined size (here 30 mm^2) was formed using the automatic dosing system of the drop tensiometer. Then, the oil droplet was placed on the bottom of the cuvette, and 600s later, contact angle was measured on the left and the right side of the water droplet. All measurements were performed at 20 °C. The experiments were conducted in triplicate.

None of the PL solutions exhibited any creeping behaviour on the glass wall, suggesting that the PL solutions were suitable for the microfluidic setup.

2.4. Microfluidic investigation of the coalescence stability

The microfluidic setup used in this study enables the analysis of droplet coalescence within tens of milliseconds after formation (31–173 ms), providing a temporal resolution that is not achievable with conventional bulk or interfacial techniques. This allows us to probe early interfacial formation that are critical for understanding emulsifier performance under rapid emulsification conditions, especially in mixed protein-PL systems where competition adsorption occurs. Additionally, the ability to perform experiments at elevated temperatures under well-defined flow conditions offers insight into the role of thermal effects during droplet formation and stabilisation. The microfluidic experiments were conducted as follows.

2.4.1. Microfluidic chips and connectors

A custom-designed borosilicate glass microfluidic chip (Micronit Microtechnologies B.V., Enschede, The Netherlands) of which the dimensions are shown in Fig. 1, was used. The design of the microfluidic device was based on (Krebs et al., 2012a; 2013). In short, the chips consist of two channels (one for the oil phase and one for the aqueous phase) that merge at a T-junction, where oil droplets are formed (Fig. 1). Next, the oil droplets individually flow through a meandering channel (1.6 mm–25.6 mm), where further emulsifier adsorption can take place. After that, the oil droplets enter a wider coalescence channel, allowing droplets to interact and possibly coalesce if not sufficiently stabilised.

The chips were placed in a chip holder and connected to a pressure controller (OB1, Elveflow, France) to inject oil (containing the PL) and aqueous phase (containing the β-LG) into the chip. The flow rate was controlled and monitored using mini CORI-Flow sensors (Bronkhorst B. V., Netherlands). The flow rate of the aqueous phase was fixed, based on (Krebs et al., 2012b; Muijlwijk et al., 2017), at 42 µl/min, while the flow of the oil phase was fixed at 2 µl.

For all measurements, the protein and PL solutions were tempered to the required temperature (either 20, 50, 60, 70, 80, or 90 °C) 30 min before the start of the experiment, and the chip was rinsed for several minutes with the tempered solutions. Additionally, a custom-designed lid, connected to compressed air and a heating unit, was placed onto the chip holder for the measurements at higher temperatures (50 °C or higher) to keep the temperature constant.

2.4.2. Imaging and data analysis

Droplet observation was done with a light microscope (Axiovert 200 MAT, Carl Zeiss B.V.) connected to a high-speed camera (MotionPro Y4-A2). One thousand images were recorded at the inlet and outlet of the coalescence channel at 30 frames per second. The two-dimensional volume of each droplet was determined using ImageJ (Muijlwijk et al., 2017). The mean droplet volume (V_f) was calculated and compared to the mean volume of non-coalesced droplets, i.e., the initial oil droplet

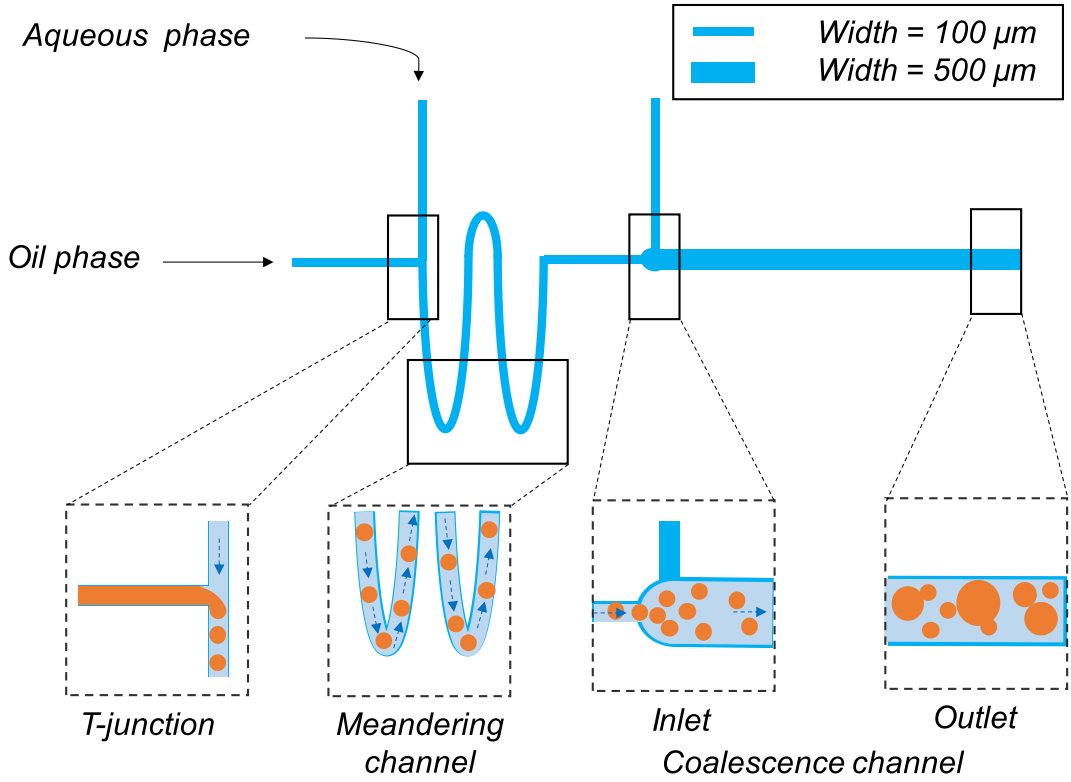

Fig. 1. Layout of the microfluidic chip with an adsorption channel length of 14.8 mm. The adsorption time was 100 ms. Other designs are depicted in the Appendix Figure S-2. Note: this design is not to scale.

volume before entering the coalescence channel (V_i). The calculated volume was based on the droplet diameter, and a pancake-like shape of the oil droplets was assumed. Since the flow conditions were kept constant for all measurements, all oil droplets had an initial diameter of around 70 μm (monomodal distribution).

The mean number of coalescence events N_{cal} follows from (E. Hinderink et al., 2025):

$$N_{coal} = \frac{V_f}{V_i} - 1 \quad (1)$$

An N_{coal} of one, indicates that, on average, each oil droplet coalesced once (average droplet volumes doubled compared to the initial volume; this value does not reflect the oil size distribution). We acknowledge that stating an average number of coalescence events (e.g. "once per droplet") does not imply a uniform doubling of droplet volume across the entire emulsion. Rather, it reflects a statistical mean across a distribution in which some droplets may not coalesce at all, while others may undergo multiple coalescence events. In this study, changes in average droplet volume were used as an indirect indicator of overall coalescence activity. We acknowledge that this simplification may mask the heterogeneity of the underlying process. For this reason, we have included representative images of the coalescence channel where appropriate.

Additionally, the frequency of coalescence events was calculated out of the mean number of coalescence events and the residence time to be able to compare the results obtained with the different chips (difference in adsorption times). The residence time inside the coalescence channel was calculated (equation (2)) from the coalescence channel length (L_{coal}) and the mean drop velocity (v_{drop})(which at a flow rate of 42 μl/min corresponds to $1.3*10^{-2}$ m/s (Krebs et al., 2012a; Muijlwijk et al., 2017);).

$$t_{res} = \frac{L_{coal}}{v_{drop}} \quad (2)$$

Accordingly, the residence time was 31 ms (meandering channel length 1.6 mm), 65 ms (meandering channel length 4.5 mm), 100 ms (meandering channel length 14.8 mm) and 173 ms (meandering channel length 25.6 mm) (supplementary material, figure S-2).

2.4.3. Statistics

The mean number of coalescence events N_{coal}, and the average and the standard deviation were calculated for each measurement.

A two-way analysis of variance (ANOVA) (using N_{coal}, standard deviation, number of replicates per group n = 1000) was performed to examine the effects of time and concentration on the number of coalescence events. Prior to conducting the ANOVA, the normality of the residuals was tested using the Shapiro-Wilk test, while the homogeneity of variances was assessed using Levene's test. It was assumed that the observations were independent.

To examine the effects of adsorption time (for same PL concentration and type), PL addition, PL type (PL 1–4) or temperature on the number of coalescence events for a fixed adsorption time, a one-way analysis of variance (ANOVA) was performed (using N_{coal}, standard deviation, number of replicates per group n = 1000). Before conducting the ANOVA, the data's normality and variances' homogeneity were tested, as mentioned above.

Significant differences between the groups were determined by a p-value threshold of 0.05. Where applicable, post-hoc pairwise comparisons were conducted using Tukey's HSD test.

Statistical analysis was performed with the Statistics and Machine Learning Toolbox of MATLAB (version R2023b).

3. Results and discussions

3.1. Individual emulsifiers at room temperature

First, we analysed the coalescence stability of oil-water emulsions stabilised by β-lactoglobulin (β-LG) for different β-LG concentrations and adsorption times (reference system 1, Fig. 2). Concentrations between 0.0001 and 0.01 wt% were used at different adsorption times to cover a range where β-LG forms stable/unstable emulsions, based on a previous study (Muijlwijk et al., 2017).

The mean number of coalescence events N_{coal} for the oil-water emulsion stabilised by 0.0001 wt% β-LG was larger than one, regardless of the adsorption time (Fig. 2). This number got significantly ($p < 0.05$) lower as the adsorption time increased but at the highest adsorption time (173 ms) coalescence still occurred ($N_{coal} = 0.6$). We can conclude that 0.0001 wt% β-LG was not able to fully stabilise the oil droplets, as expected from previous work (Muijlwijk et al., 2017).

Increasing the β-LG concentration significantly decreased N_{coal} ($p < 0.05$), and at concentrations of 0.001 wt% or higher, combined with adsorption times of 65 ms or more, N_{coal} dropped below 0.4. This suggests that coalescence was effectively suppressed under these conditions, with longer adsorption times further enhancing droplet stability (Fig. 2). Statistical analysis (Tukey's HSD) revealed that both concentration and adsorption time significantly affected coalescence events ($p < 0.05$), with these effects being interdependent. It seems that an adsorption time of 65 ms was sufficient for 0.001 wt% β-LG to prevent coalescence. In previous studies this was related to the theoretical monolayer coverage of β-LG (1.7 mg/m^2), which at the given conditions should already be reached at ~0.006 wt % β-LG (Muijlwijk et al., 2017; Tcholakova et al., 2002).

Next, we investigated the coalescence stability of droplets stabilised by different PLs (reference systems 2, Fig. 3). A fixed PL concentration of 0.005 wt% was used, as we have shown that at this concentration, full surface coverage at the oil-water interface was reached in emulsions (Risse, Bridot, Yang, et al., 2025b). The results for PC 18:1 (large headgroup, unsaturated FA chain) as a function of adsorption times (31 ms–173 ms) are shown in Fig. 3. The results for the other PLs can be found in the supplementary material (Figures S-3).

As expected, N_{coal} was highest for 31 ms and lowest for 173 ms, and always larger than two for all adsorption times tested (Fig. 3, 31ms to 173 ms), as is the case for the other PLs (Figures S-3). The low stability was mainly a result of the low PL concentration, resulting in slow adsorption and poor initial interfacial coverage (Hildebrandt et al., 2018; Shchipunov & Kolpakov, 1991). By increasing the adsorption time, the number of coalescence events slightly reduced (N_{coal} of 10.5 to 2.8). The reduction in coalescence events was significant; with significant differences between the groups ($p < 0.05$) and within the groups (except for 31 ms and 65 ms). Nevertheless, the overall conclusion is that at the concentration tested, the PLs did not stabilise the droplets, not even at the longest absorption time of 173 ms, which was severely due to limited solubility of the PL (Hildebrandt et al., 2018; Shchipunov & Kolpakov, 1991).

In comparison, in emulsion production, PL concentrations of around 1 wt% total weight were needed for stable emulsions (Andersson et al., 2025; Kabalnov et al., 1996). In addition, the PLs were dissolved in the dispersed (oil) and not the continuous phase. The low coalescence stability was, thus, expected based on their solubility (Bancroft's rule) (Davis, 1994; Ravera et al., 2021; Silva & Loh, 2022) and the low HLB value, which would make the PLs more suitable to stabilise a water-in-oil emulsion, when used alone (Hong et al., 2018; Kralova & Sjöblom, 2009; Premlal Ranjith & Wijewardene, 2006; Rahaman et al., 2022).

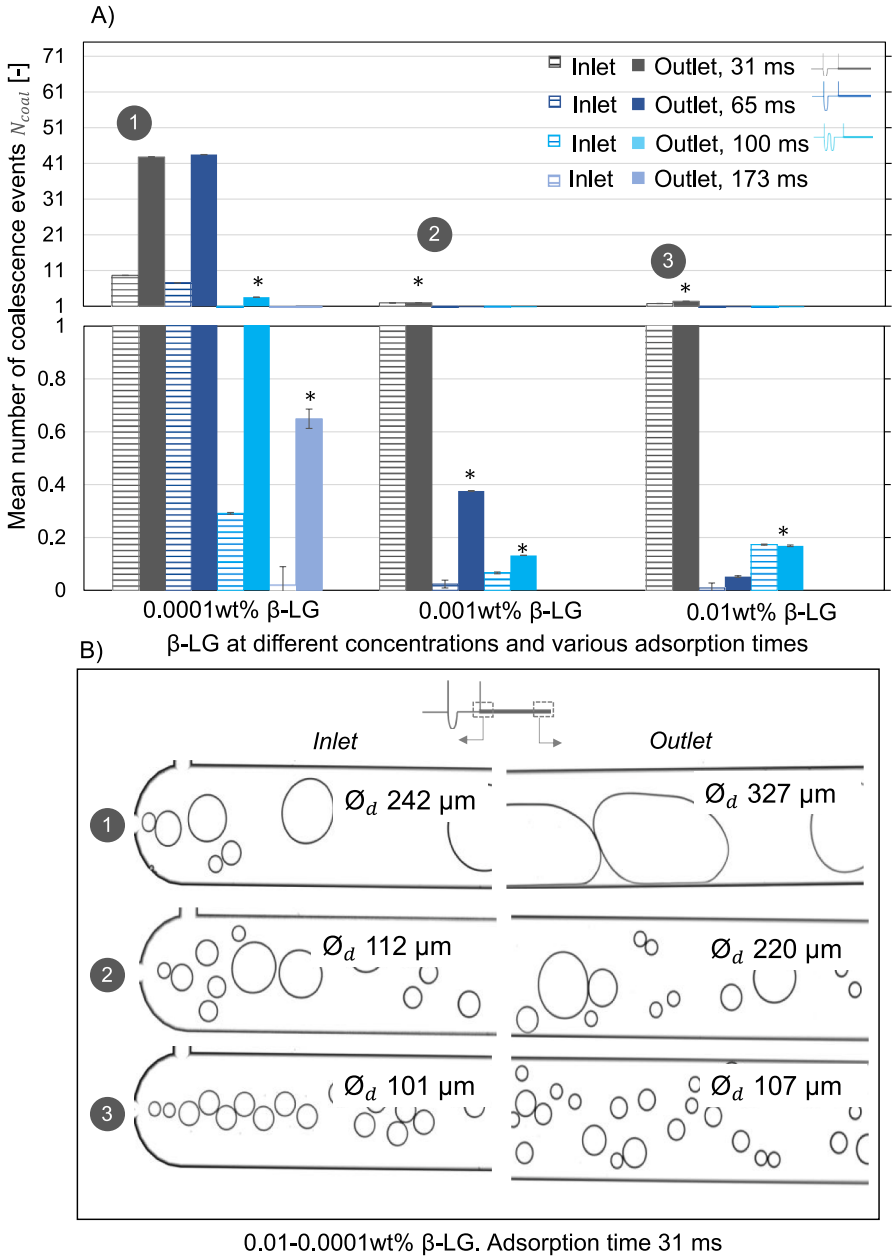

Fig. 2. A) Mean number of coalescence events N_{coal} of oil droplets stabilised by β-LG at various β-LG concentrations (0.0001–0.01 wt%) and various adsorption times (31–173ms). B) shows a representative image of the inlet and outlet of the coalescence channel with the 0.0001 wt%, 0.001 wt% and 0.01 wt% β-LG emulsions (top to bottom) at a fixed adsorption time of 31 ms. The temperature was fixed to 20 °C. $Ø_d$: average diameter (µm). The initial diameter, i.e., the diameter before entering the coalescence channel, was ~70 µm in all cases. For results at room temperature, see Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

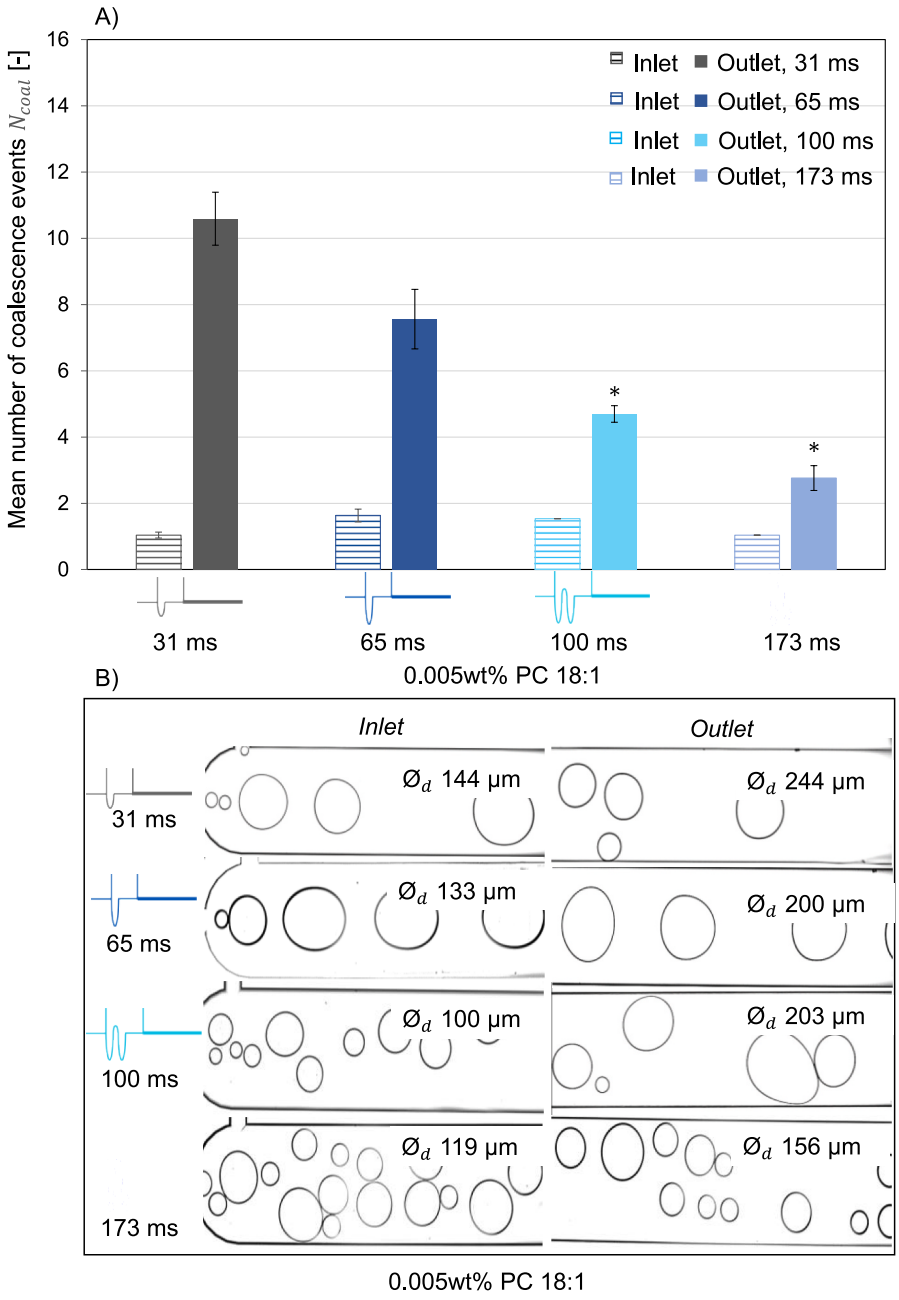

Fig. 3. A) Mean number of coalescence events N_{coal} of oil droplets stabilised by 0.005 wt% PC 18:1 and various adsorption times (31ms–173ms). B) shows representative images of the inlet and outlet of the coalescence channel. The temperature was fixed to 20 °C. $Ø_d$: average diameter (µm). The initial diameter, i.e., the diameter before entering the coalescence channel, was ~70 µm in all cases. * means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

3.2. Individual emulsifiers at higher temperatures

Next, we analysed the coalescence stability of β-LG or PL stabilised emulsions as a function of temperature (T 50–90 °C) before investigating their combined effects.

In the following, we describe the impact of temperature on β-LG stabilised emulsions at an adsorption time of 65 ms (Fig. 4). N_{coal} progressively increased with temperature, and the order of magnitude is relatively low (below 0.45), with the difference between the groups being significant ($p < 0.05$). At around 70 °C, oil droplet aggregation started to take place, albeit without affecting the number of coalescence events since the oil droplets did not merge. Apparently, the interactions between proteins became stronger at elevated temperatures, both at the interface (in-plane interactions) and inter-droplet (Bernard et al., 2011; Dissanayake & Vasiljevic, 2009; D. A. Kim et al., 2005; Moro et al., 2001, 2013). For instance, the strength of the repulsion between groups of equal charges weakens with the temperature (Folch et al., 2010; Pucci & Rooman, 2017), increasing the risk of oil droplet aggregation when droplets approach one another.

Heat-induced structural changes in the protein are expected to have played a role as well, given the denaturation temperature of β-LG that is ~70 °C (see supplementary material, Figure S-5). Above this temperature, β-LG unfolds and interacts differently, which is expected to contribute to flocculation and aggregation of oil droplets possibly by a bridging effect (Euston et al., 2002, 2009; Liang et al., 2013, 2017; McClements, 2004; McSweeney et al., 2004). Interestingly, the oil

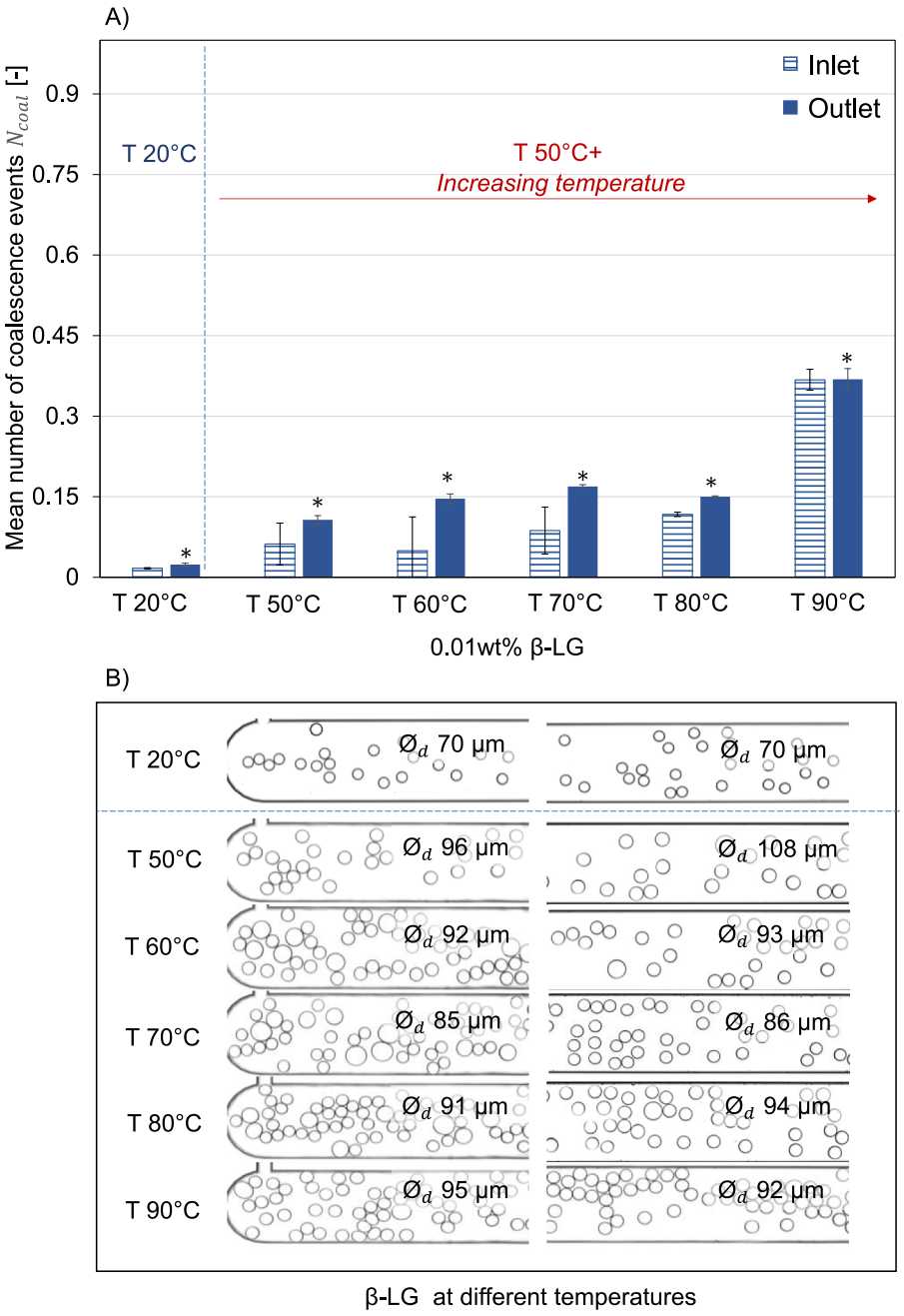

Fig. 4. A) Mean number of coalescence events N_{coal} of droplets stabilised by 0.01 wt% β-LG at a fixed adsorption time of 65 ms. B) shows representative images of the inlet and outlet of the coalescence channel with the β-LG stabilised emulsions. The temperatures used are 20, 50, 60, 70, 80, and 90 °C. For β-LG, severe oil droplet aggregation was observed at temperatures around 70 °C, which is not reflected in the calculated number of coalescence events since the droplets did not merge. Please note the smaller y-axis compared to the previous figures. This is due to the significantly lower coalescence events. The initial diameter, i.e. the diameter before entering the coalescence channel, was ~70 µm in all cases. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

droplets did not coalesce much after aggregation, which is indicative of the formation of robust interfacial layers. When two droplets encounter each other (due to flow-induced forces), the interface will flatten, forming a film of continuous phase which drains until reaching a certain critical thickness. Aggregation between droplets is reversible as long as the film thickness remains above a critical value (usually around 50–100 nm, common thin film) (Walstra, 2002). However, if the film thickness drops below a critical value (typically ~3–4 nm), irreversible coalescence occurs (Ravera et al., 2021; Tarazona et al., 2012). Whether a film thins sufficiently to break depends on the interfacial layer properties, in particular the elasticity (Botti et al., 2022, pp. 1423–1434; Dickinson, 1998; Dickinson et al., 1988; E. B. A. Hinderink et al., 2020b; Langevin, 2025; Mezger, 2010; Rühs et al., 2012). For the droplets investigated here, the adsorbed β-LG seems to have formed a protective viscoelastic shell around the droplets.

Next, we studied the impact of the temperature on the coalescence of droplets stabilised by PC 18:1. We focused on adsorption times above 100 ms as the PC 18:1-stabilised emulsions were highly unstable at shorter timescales. The results for 100 ms adsorption time are shown in Fig. 5A and for 173 ms in Fig. 5B.

N_{coal} was larger than one regardless of the adsorption time or temperature, with the absolute value decreasing when going from 20 to

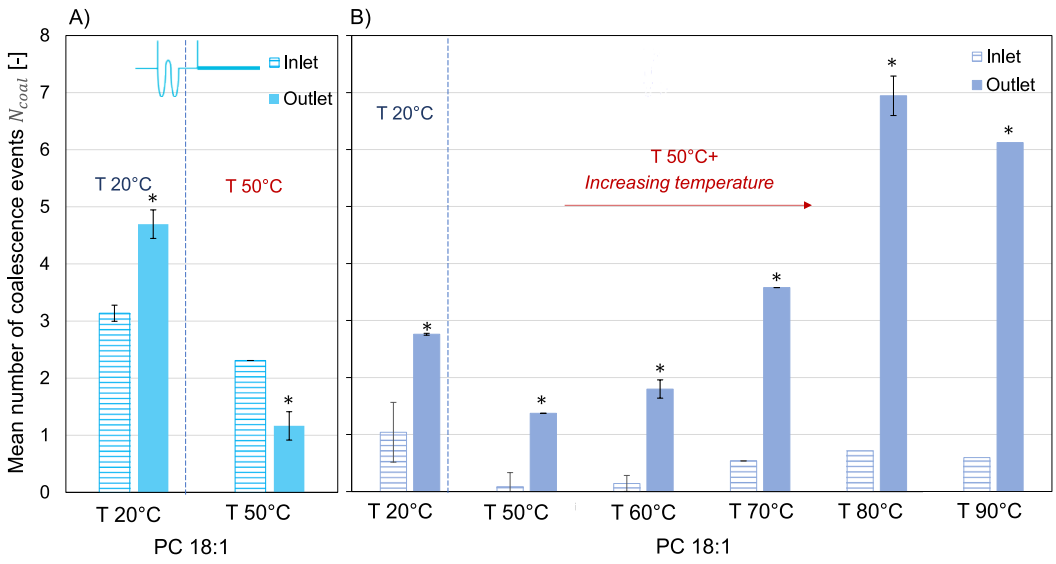

Fig. 5. Mean number of coalescence events N_{coal} of droplets stabilised by PC 18:1 at adsorption times of 100 (A) and 173 ms (B). The temperatures used are 20, 50, 60, 70, 80, and 90 °C. PC 18:1: unsaturated phosphatidylcholine. Measurements at room temperature, see Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

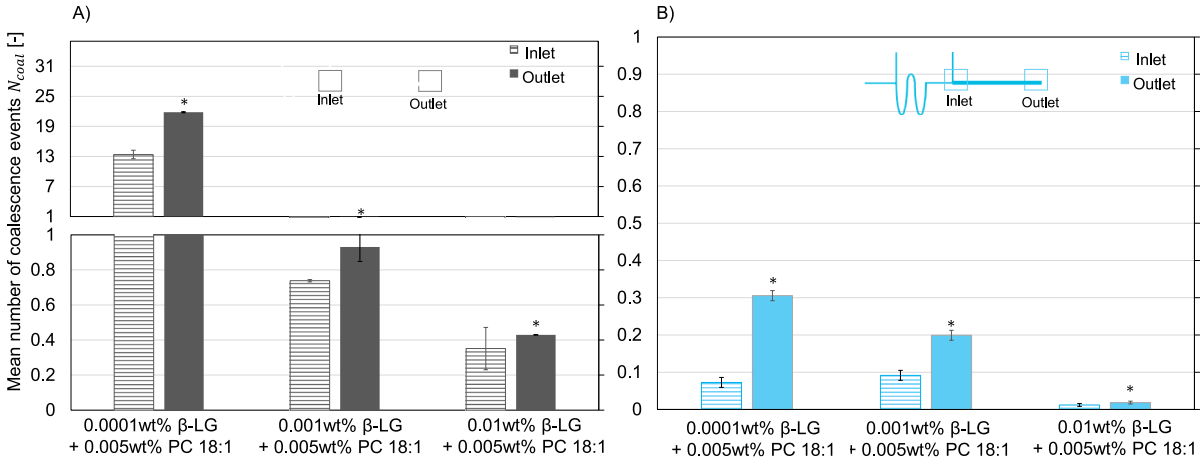

Fig. 6. Mean number of coalescence events N_{coal} of oil-water droplets stabilised by β-LG + PC 18:1 at various β-lactoglobulin concentrations (0.0001 wt%-0.01 wt%) and an adsorption time of 31 (A) and 100 ms (B). The temperature was 20 °C. Please note the scale of the y-axis compared to the previous figures. This is due to the significantly lower values of coalescence events. β-LG: β-lactoglobulin. PC 18:1: unsaturated phosphatidylcholine. measurements at room temperature, see Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

50 °C (Fig. 5A and B), most probably due to the PL's faster molecular movement (resulting in faster adsorption) combined with a lowered viscosity of the oil (leading to a faster incorporation of the PL into the interfacial layer) (Ivanova et al., 2020; Li & Liu, 2024). At higher temperatures (70 °C or higher, see Fig. 6 B), N_{coal} was considerably higher (significant difference between the group $p < 0.0001$), possibly due to a phase transition of the PL from a liquid-ordered (i.e. liquid-crystalline) to liquid-disordered state at the interface (Leonenko et al., 2004). For PLs it has been shown that the interfacial layer structure changes once a phase transition temperature has been reached (Dietrich et al., 2001; González-Henríquez et al., 2017; K. Kim et al., 2011; Pichot et al., 2013). Below the liquid-crystalline to liquid-disordered phase transition temperature (<50 °C), the PL will pack tightly at the interface to form ordered structures, but once a phase transition from ordered to disordered occurs, disordered domains at the interface with reduced interfacial thickness form (Dietrich et al., 2001; González-Henríquez et al., 2017; Leonenko et al., 2004; Tristram-Nagle et al., 1998). In addition, in a previous study, we showed that weaker in-plane interactions between adsorbed surfactant molecules occur above their melting point, causing higher mobility of the surfactant (Risse & Drusch, 2024). In the emulsion system, this would make the interfacial layer more prone to rupture during the thin film phase, favouring droplet coalescence. A similar effect of temperature on coalescence was recently reported for Tween and SDS-stabilised oil-water droplets observed with microfluidics. The authors linked this to a stronger perturbation in the thin aqueous film separating the droplets, leading to faster film drainage (Bera et al., 2021). Apparently, the heat-induced reduction in coalescence stability of surfactant stabilised emulsions is a combination of different factors, involving the change of viscosity of the fluid phases (dispersed oil phase and continuous phase) and an altered interfacial behaviour of the surfactant once the phase transition temperature is reached.

3.3. Combined β-lactoglobulin and phospholipid at room temperature

After the coalescence stabilities of emulsions stabilised by the individual emulsifiers were charted, we studied the combined effects of emulsifiers at room temperature. We first tested the effect of different ratio of β-LG and PC 18:1 on droplet coalescence for an adsorption time of 31 or 100 ms at β-LG concentrations of **0.01**–**0.0001** wt% and a fixed PL concentration of 0.005 wt% (Fig. 6).

For β-LG + PC18:1 stabilised emulsions N_{coal} was below one for adsorption times of 31 ms as long as the β-LG concentration was **0.001** wt% or higher (Fig. 6 A). As a comparison, for adsorption times of 31 ms, the purely β-LG stabilised emulsions had N_{coal} values of one or larger, even at the highest β-LG concentration (**0.0001** wt% β-LG $N_{coal} = 42.8$ and **0.01** wt% β-LG, $N_{coal} = 2.4$, Fig. 2). This indicates that using a blend of protein and PL significantly reduced coalescence compared to the individual components ($p < 0.05$), hinting at a synergistic effect. Two different explanations were found in literature albeit for long time-scales **(1)** The presence of PL allows a faster interfacial stabilisation, meaning that the simultaneous adsorption of protein and PL favours the formation of a mixed interfacial layer that increases interfacial stiffness (Münch et al., 2024a; Shen et al., 2023; Yan et al., 2025; Yesiltas et al., 2019a). In addition, strong repulsive forces (e.g., steric or electrostatic) may contribute to droplet stability by reducing the risk of two droplets approaching each other close enough for coalescence to occur (Dickinson, 1994, pp. 59–74; Gomes et al., 2018; E. B. A. Hinderink et al., 2019; Ho et al., 2018; Ji et al., 2015; McClements, 2005; McClements & Gumus, 2016). **(2)** The use of mixed emulsifiers has been linked to higher droplet stability due to protein PL complexation or multilayer formation at the interface, which makes the interfacial more elastic and, thus, less prone to rupture (Bylaite et al., 2001; Dickinson & Yamamoto, 1996; He et al., 2008; McClements & Jafari, 2018; Sünder et al., 2001; Waninge et al., 2005; Yesiltas et al., 2019b; M. Zhang et al., 2022). Since no β-LG PL interaction was found for unsaturated PLs (Bos & Nylander, 1996; Cornell & Carroll, 1985; Kristensen et al., 1997; Lefèvre & Subirade, 1999, 2000; Lookman et al., 1982; Risse, Bridot, Bäther, et al., 2025a), the increased droplet stability observed here is more likely an effect of simultaneous adsorption of the two emulsifiers rather than protein PL interaction. The simultaneous adsorption of two emulsifiers can enhance droplet stability due to several synergistic interfacial effects, such as faster interfacial coverage (co-adsorption of protein and PL) and the formation of a mixed interfacial layer. Several authors have linked such mixed interfacial layer to increased interfacial stiffness, resulting from tighter molecular packing, changes in protein conformation, resulting in more stable emulsions (Dickinson, 2011; Dickinson & Yamamoto, 1996; Heiden-Hecht & Drusch, 2022; McClements & Jafari, 2018; Y. Zhao et al., 2024). We acknowledge that competitive adsorption between these two species could theoretically interfere with protein adsorption. However, at the short timescales investigated in this study, interfacial concentrations are assumed to be sufficiently low such that competition for interfacial area is not yet a limiting factor. Competitive displacement effects are more likely to become relevant once a certain degree of interfacial coverage is reached (Bai & McClements, 2016; Bos & van Vliet, 2001; Cornell & Patterson, 1994; Courthaudon, Dickinson, Matsumura, et al., 1991; Mackie et al., 2001; Münch et al., 2024b; Nylander et al., 2019a).

At **0.0001** wt% β-LG, droplet coalescence occurred ($N_{coal} > 10$, t = 31ms, Fig. 6 A). This means that an adsorption time of 31 ms was either not enough for the interfacial layer to form, or the amount of emulsifier was simply not enough to cover the interface completely. At higher concentrations (≥0.001 wt%), even at adsorption times as short as 31 ms (Fig. 6 A), the droplets were very stable, suggesting a very fast synergistic effect. We repeated the experiment for an adsorption time of 100 ms (β-LG concentrations of 0.01–0.0001 wt%) to test if there was a time dependency (Fig. 6 B), and that was the case for the mixture. Even at the lowest β-LG concentration of 0.0001 % (Fig. 6 B), droplets were stable, while droplets stabilised with the single components were not (Figs. 2 and 3).

Next, we studied the impact of the PL's structure on droplet stability when using β-LG + PL (*PC 18:0* (large headgroup, saturated FA chain), *PC 18:1* (large headgroup, unsaturated FA chain), *PE 18:0* (small headgroup, saturated FA chain) or *PE 18:1* (small headgroup, unsaturated FA chain in combination) at different concentrations and a fixed adsorption time of 31 ms (Fig. 7).

The stability of β-LG + PL stabilised droplets was considerably smaller than that of the individual components (Fig. 7 vs. Fig. 2), even at **0.001** wt% β-LG (Fig. 7 A). Although one-way ANOVA and post-hoc pairwise comparison suggests significant differences ($p < 0.05$) between the N_{coal} values of **0.01** wt% *β-LG + saturated PL* and 0.01 wt% *β-LG + unsaturated PL* (Fig. 7 B), differences in droplet stability were small ($N_{coal} < 1$). This was in particular the case for 0.01 wt% β-LG + PL (Fig. 7 B), suggesting that the synergistic effect described above is not limited to a specific PL but a general feature. Based on a previous study

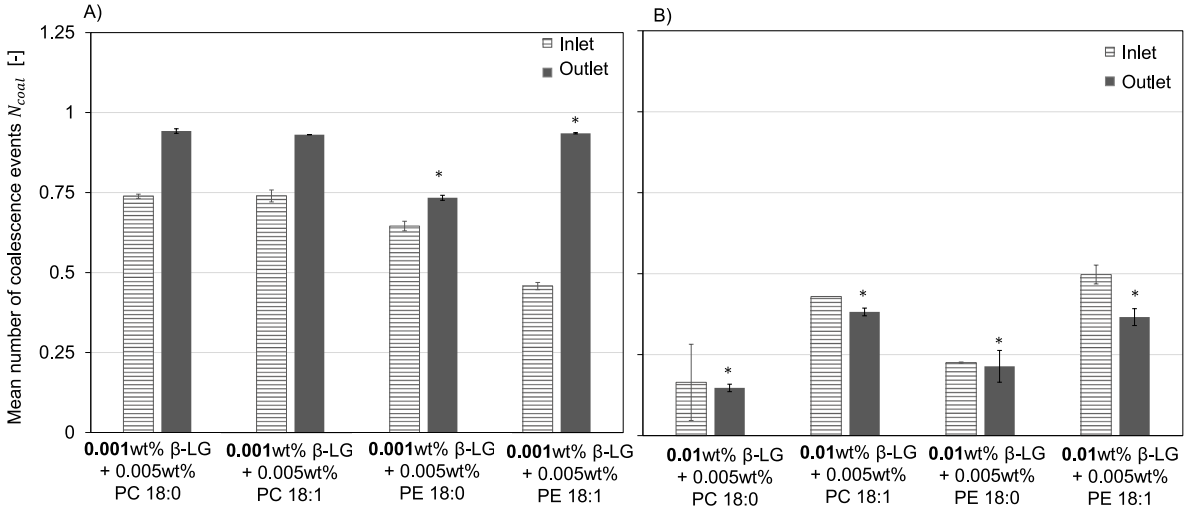

Fig. 7. Mean number of coalescence events N_{coal} of oil-water emulsions stabilised by β-LG and various PL at fixed adsorption time of 31 ms. β-LG concentration was 0.001 wt% (A), 0.01 wt% (A). PC 18:0: saturated phosphatidylcholine. PC 18:1: unsaturated phosphatidylcholine. PE 18:0: saturated phosphatidylethanolamine. PE 18:1: unsaturated phosphatidylethanolamine. Results for room temperature in Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

in which interfacial layer stability was investigated, it was expected that the combination of *β-LG + unsaturated PL* would reduce the emulsion stability, while *β-LG + saturated PL* would enhance droplet stability (Risse, Bridot, Yang, et al., 2025b). For the microfluidic experiments, that is not the case. All PLs had a molecular weight between 750 and 850 Da, probably resulting in similar diffusivity and adsorption kinetics at short timescales. Apparently, the interfacial concentration of the PL milliseconds to seconds after drop formation was not high enough to push the protein out of the interface and, consequently, co-existence with the protein was possible in all four cases (Bai & McClements, 2016; Bos & van Vliet, 2001; Cornell & Patterson, 1994; Mackie et al., 2001; Münch et al., 2024a; Nylander et al., 2019b). Taken together, the findings of our previous study on interfacial rheology, where we observed that the displacement of β-LG by unsaturated PLs was not immediate but rather a time-dependent process that became noticeable only after several minutes, and the results of the present microfluidic study, lead us to the following hypothesis:**(1)** the displacement of proteins does not occur within the first milliseconds of emulsification. **(2)** PL did not hinder the β-LG from adsorbing, as this would have lowered the coalescence stability due to the reduced interfacial viscoelasticity (Bos & van Vliet, 2001; Mackie, 2004; McClements & Jafari, 2018; Nylander et al., 2019a). And **(3)** formation of a viscoelastic protein + saturated PL network requires time and or is temperature dependent.

To conclude, at room temperature and on these short time scales, both components contribute to interface stabilisation. The presence of PL allows for faster interfacial stabilisation, and leads to higher droplet stability regardless of the PL type.

3.4. Combined β-lactoglobulin phospholipid at elevated temperatures

Finally, we analysed droplet stability for mixtures of β-LG + PL as a function of temperature. Since the molecular structure did not seem to impact droplet stability significantly ($p < 0.05$) at room temperature, we focused on PC 18:1 (large headgroup, unsaturated FA chain), and later compare with PC 18:0 (large headgroup, saturated FA chain) since these PL show the greatest difference in their phase behaviour (PC 18:0 solid to liquid phase transition at 55 °C (Chen et al., 2018) vs. −15 °C in the case of PC 18:1 (Klacsová et al., 2016; Pentak, 2014)).

To begin with, we tested β-LG at **0.0001-0.01** wt% in combination with PC 18:1 at an adsorption time of 31 ms and temperature of 50 °C (Fig. 8 A). At 50 °C, N_{coal} was below one for all β-LG + PC 18:1 emulsions, even at 0.0001 wt% β-LG concentration (Fig. 8 A, $N_{coal} = 0.8$), unlike at 20 °C, where 0.001 wt% β-LG + PC 18:1 droplets were unstable at adsorption times of 31 ms (Fig. 6 A). Since neither 0.0001 wt% β-LG (supplementary material, Figure S-6) nor PC 18:1 alone (Fig. 3) led to stable droplets at 50 °C, the increased stability needs to be caused by a synergistic effect; for example the PL's faster diffusion and adsorption at a lower viscosity oil interface, as described earlier.

To test if this synergistic effect could also lead to increased coalescence stability at temperatures, which are above the protein's denaturation temperature and possibly above the phase transition temperature of the PLs, we increased the temperature gradually from 50 to 90 °C, for **0.001** wt% β-LG in conjunction with PC 18:1 (Figure S-7). The oil droplets started to stick to the channel wall at the inlet once a temperature of 70 °C was reached, and began to coalesce. These droplets are expected to have low surface coverage and thus, less effective protection against coalescence, especially when the contact time between droplets is extended due to sticking. For this reason, we increased the β-LG concentration to **0.01** wt% and studied the effect of temperature in combination with PC 18:1 (Fig. 8 B).

For **0.01 wt**% β-LG + PC 18:1, N_{coal} was below 0.5 over the whole temperature range (50–90 °C, Fig. 8 B); the droplets showed high stability towards coalescence. The severe oil droplet aggregation at 70 °C observed for β-LG only was absent when used in conjunction with PC 18:1, and coalescence was reduced considerably compared to protein only. It has been reported that above a characteristic temperature (>50 °C), the PL exhibits expanded behaviour at the interface (Dietrich et al., 2001; Tristram-Nagle et al., 1998) and appears to mix homogeneously with proteins, while at room temperature, the PL exhibit a condensed behaviour and segregates from proteins in mixed monolayers (Cornell & Carroll, 1985). The increased stability of the β-LG + PC 18:1 emulsion compared to the purely β-LG stabilised emulsion might, therefore, be related to the presence of PC 18:1 at the interface, which might contribute to electrostatic or a steric repulsion, thereby reducing the risk of oil droplet aggregation (Nylander et al., 2019b). Additionally, the presence of PC 18:1 appears to enhance the interfacial packing density (Dietrich et al., 2001; Nylander et al., 2019a), thereby increasing interfacial viscoelasticity.

Finally, we analysed if the saturation of the PL FA chain correlates with droplet stability when using β-LG and PL. We compared *PC 18:1*

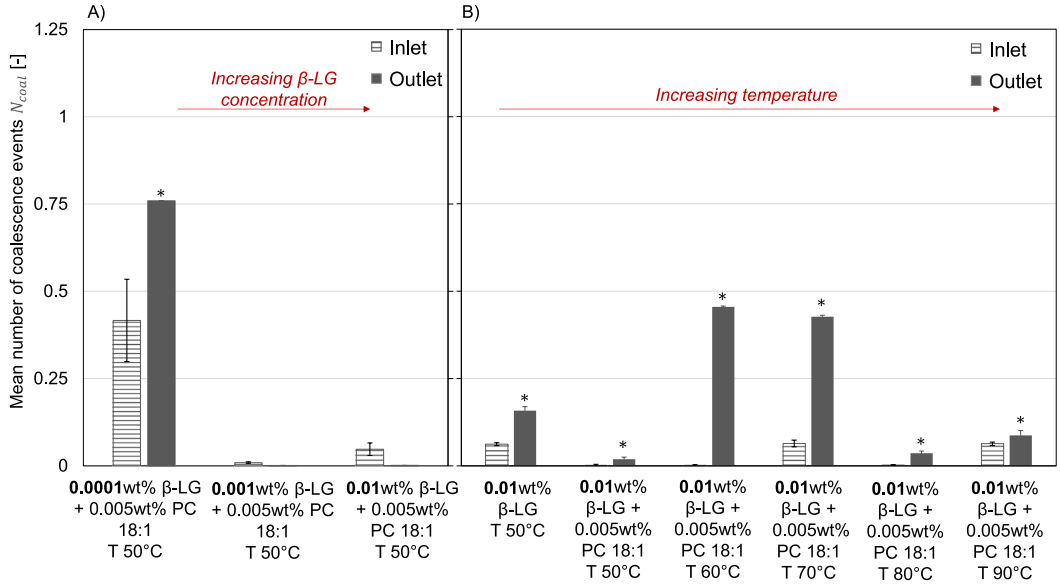

Fig. 8. Mean number of coalescence events N_{coal} of droplets stabilised by β-LG + PC 18:1 (B) at various β-LG concentrations (0.0001–0.01 wt%) and a fixed adsorption time of 31 ms. The temperature was 50 °C in A) and varied from 50 °C (2), 60 °C (5), 70 °C (7), 80 °C (8) and 90 °C (9) in B). For measurements at room temperature, see Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

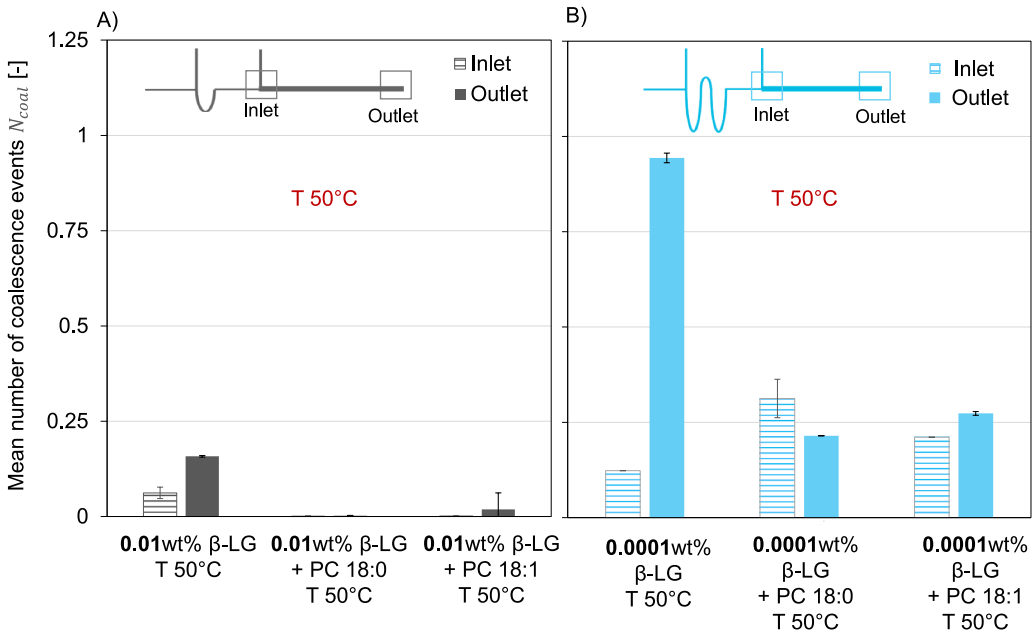

Fig. 9. Mean number of coalescence events N_{coal} of droplets stabilised by β-LG + PC 18:0 (1, 3) or β-LG + PC 18:1 (2, 4) at an adsorption time of 31 ms (A) and 100 ms (B). The temperature was either 20 °C or 50 °C. β-LG: β-lactoglobulin. PC 18:1: unsaturated phosphatidylcholine. PC 18:0: saturated phosphatidylcholine. Results for room temperature, see Fig. 3. *means significant differences between this group and all the other groups according to post-hoc pairwise comparisons ($p < 0.05$).

and *PC 18:0* (Fig. 9), because these have the greatest differences in their melting points. In particular, saturated PLs have higher melting points (PC 18:0 solid to liquid phase transition at 55 °C (Chen et al., 2018) vs. −15 °C in the case of PC 18:1 (Klacsová et al., 2016; Pentak, 2014) and may form crystalline networks at the interface already at room temperature, which is not the case for unsaturated PL (Risse, Bridot, Yang, et al., 2025b).

For both 0.01 % β-LG mixtures with *PC 18:1* and *PC 18:0*, N_{coal} values were below 1 (Fig. 9A), with significantly lower values ($p < 0.05$) for blends compared to the β-LG emulsions. Similar effects were found at 100 ms for 0.0001 wt% β-LG.

Next, we increased the system's temperature from 50 °C to 90 °C also for *the β-LG + PC 18:0* blend. Severe sticking occurred when using *PC18:0* with 0.01 wt% β-LG at temperatures above 75 °C (supplementary material, Figure S-8), while this was not the case for *the β-LG + PC 18:1* blend (data for PC 18:1 see Fig. 8). This leads us to conclude that β-LG + PL interactions depend on the PL's FA chain (Bos & Nylander, 1996; Kasinos et al., 2013; Kristensen et al., 1997; Lefèvre & Subirade, 1999, 2000; Nylander et al., 2019b; Risse, Bridot, Yang, et al., 2025b) and the state the PL is in (solid or liquid, ordered vs. disordered) (Dietrich et al., 2001; González-Henríquez et al., 2017; K. Kim et al., 2011; Pichot et al., 2013).

It has been reported that saturated PL interacts with β-LG either electrostatically, hydrophobically or via hydrogen bonding, while no β-LG PL interaction was found for unsaturated PLs (Bos & Nylander, 1996; Cornell & Carroll, 1985; Kristensen et al., 1997; Lefèvre & Subirade, 1999, 2000; Lookman et al., 1982; Risse, Bridot, Bäther, et al., 2025a), which has been related to the bend in the molecule that sterically hinders the PL from binding onto β-LG (Bos & Nylander, 1996). These interactions have primarily been observed in aqueous phase (Bos & Nylander, 1996; Cornell & Carroll, 1985; Lookman et al., 1982) and at the air-water interface (Heckl et al., 1987; Junghans et al., 2010; Saint-Pierre-Chazalet et al., 1992; X. Zhang et al., 2007), rather than at the oil–water interface. Nonetheless, we believe that the key difference lies not in whether such interactions occur, but in the extent to which they contribute to interfacial structure and, ultimately, to emulsion stability. Based on previous studies it was expected, that β-LG + saturated PL emulsions (*PC 18:0*) show a greater stability than β-LG + unsaturated PL (PC 18:1) – especially at higher temperatures due to these β-LG + saturated PL interactions (>70 °C). This is because β-LG PL interactions may shift the unfolding of β-LG to higher temperatures, thus possibly enhancing the heat stability of emulsions as reported by (Bos & Nylander, 1996; Kristensen et al., 1997). In the similar way, the thermotropism of phospholipids may be altered by interactions with β-LG (Lefèvre & Subirade, 1999). For PC 18:0 it has been reported that the PL is in a solid state for temperatures up to 55 °C, after which a phase transition to the liquid-crystalline (Lα) phase (Chen et al., 2018). Interactions between β-LG + PC 18:0 might push the solid to liquid phase transition (chain melting point) towards higher temperatures. This would increase the emulsion stability since the interface shows predominantly elastic properties till the PL is in a liquid state (Risse & Drusch, 2024) (see section 2.3).

Nevertheless, emulsion stability was reduced in the case of *β-LG + PC 18:0* blend compared to the *β-LG + PC 18:1* blend once the system's temperature was increased to >70 °C. The changes that we notice here in droplet stability, seem to be linked to denaturation temperature of the protein at temperatures higher than 70 °C. Below this temperature (<70 °C) a more connected protein network is present at the interface, leading to enhanced emulsion stability (Dickinson & Yamamoto, 1996; He et al., 2008; Risse, Bridot, Bäther, et al., 2025a; Waninge et al., 2005). However, once the denaturation of β-LG occurs (≥75 °C), interactions between β-LG at the interface and in the bulk may be enhanced (Dickinson & Matsumura, 1991; Monahan et al., 1993; Sengupta & Damodaran, 2000; Zhai et al., 2011), possibly contributing to flocculation and aggregation of droplets as described for the pure β-LG emulsions at higher temperatures (see section 3.2). Apparently, mixtures of β-LG + saturated PL only show beneficial synergistic effects at temperatures below the denaturation temperature of the protein and the chain melting point of the saturated PL. Interestingly, PL with unsaturated FA chains seem to partly hinder protein folding. One possible explanation is that PLs with unsaturated FA chains tend to form more disordered and loosely packed interfacial layers due to the presence of cis-double bonds, which introduce kinks in the hydrophobic tails. This results in reduced lateral packing density and weaker van der Waals interactions at the interface (Ballweg et al., 2020; Luceros et al., 2008; Martinez-Seara et al., 2008; Parks et al., 2000; Smaby et al., 1997). As a

result, the local interfacial space left for the protein may not be sufficient to induce conformational rearrangement or "unfolding" compared to interfaces formed by solely β-LG or β-LG + saturated phospholipids. Instead, the protein may remain in a more native-like or less extended conformation, which could limit its ability to interact with the non-adsorbed (bulk) protein. Further research is required to proof this.

4. Conclusions

We studied the coalescence stability of oil droplets stabilised by β-lactoglobulin, phospholipids, and combinations thereof at temperatures between 20 and 90 °C using a microfluidic technique that allows fast observations (31–173 ms). Phosphatidylethanolamine PE, and phosphatidylcholine PC, with different chains (C18:0 or C18:1) were used to investigate whether the properties of the phospholipid contribute to droplet stability. This study makes two key contributions: First, it provides new insights into the co-adsorption behaviour of PLs and proteins at oil-water interfaces on very short timescales, highlighting their synergistic role in accelerating interfacial film formation during emulsification. Second, we demonstrate how temperature during emulsification affects adsorption kinetics and how this, in turn, correlates with droplet stability, even at elevated temperatures up to 90 °C.

Droplets stabilised by β-lactoglobulin are more stable than those with phospholipids only, while combining these components always leads to enhanced droplet stability. Simultaneous adsorption of β-lactoglobulin and phospholipid leads to faster interfacial stabilisation, making the interfacial layer less prone to rupture. At the time scales that were investigated, these effects occurred irrespective of the phospholipid used, with saturated PLs interacting with the β-lactoglobulin at the interface, allowing them to coexist (leading to stiffer interfaces). In previous interfacial rheology studies, we observed that unsaturated PL tends to replace the protein from the interface (leading to weak, PL-dominated interfaces). We, therefore, conclude that short-time scales are needed to evaluate stable droplet formation, intermediate times for competitive displacement, and long-time scales to analyse the complex interfacial structure that, in turn, determines the storage and process stability of the emulsion.

Temperature affects interface stabilisation, destabilisation of films between droplets, the structure of proteins, the phase behaviour of PLs, and possibly also protein + PL interactions, and all these aspects influence droplet stability. For PL, below a critical temperature (>50 °C, phase transition from solid to liquid crystalline), droplets stability was significantly increased with increasing temperature due to the faster molecular movement. However, above this temperature, droplet stability was significantly reduced due to the change of viscosity of the fluid phases (dispersed oil phase and continuous phase) and an altered interfacial behaviour of the surfactant (liquid crystalline to liquid disordered phase transition). Similarly, the denaturation of β-LG seems connected to droplet aggregation, not necessarily coalescence, and when used in conjunction with saturated PL, aggregation was enhanced, unlike unsaturated PL.

From the experiments carried out here, clues can be derived for producing stable emulsions at a large scale, as well as the processes that can be used to create them. As mentioned, the temperature is a multifaceted factor that needs to be chosen with care depending on the components used. Amongst others, when the emulsification temperature is below the denaturation temperature of the protein, PL with saturated fatty acyl chains should be chosen to enhance the interfacial viscoelasticity by protein + PL interactions. However, when the emulsification temperature is above the denaturation temperature of the protein, PL with unsaturated fatty acyl chains should be chosen to avoid protein folding.

For emulsion design, all these aspects need to be put into the right perspective, as will be done in future studies. While the microfluidic experiments in this study provide insight into early-stage interfacial adsorption dynamics during emulsification, it is important to note that interfacial composition and properties continue to evolve after droplet formation. To complement the current findings, ongoing work in our group is focused on analysing how these time-dependent interfacial changes affect the long-term stability and functionality of emulsions, also as a function of PL type and temperature.

CRediT authorship contribution statement

Kerstin Risse: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Fathinah I. Hasyyati:** Writing – review & editing, Methodology. **Emma Hinderink:** Writing – review & editing, Methodology, Conceptualization. **Karin Schroën:** Writing – review & editing, Methodology, Conceptualization.

Declaration of competing interest

The authors have declared that no competing interest exist. This manuscript has not been published and is not under consideration for publication in any other journal. All authors approve this manuscript and its submission to the Journal of Food Hydrocolloids.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2025.111810.

Data availability

Data will be made available on request.

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