Supplementary Information (SI) for Soft Matter.
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Supporting information

What makes oil-in-water emulsions with pea protein stable? The role of excess protein in network formation and yield stress development

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1. Confocal microscopy of stable emulsion compositions with 7.5% w/v protein

To evaluate the storage stability of the prepared emulsions, the samples were kept at 19 °C for 1 week. Micrographs of oil-in-water emulsions of samples after storage in Figure S1 show a similar droplet distribution and size for samples compared to fresh emulsions in Figure 1. The average radii are listed in Table 2 in the main text. The droplet size was estimated by droplet area coverage in the images. The droplet radius was calculated based on the assumption of spherical droplets. We note that the droplet radius is a number average and that this should be kept in mind when comparing with droplet size measured in other ways. The calculations are average of three measurements and are based on the micrograph analysis presented in Figure S2 and Figure S3. Several works have been perfomed within the stereology field to convert an area particle size obtained from a thin slice image (similar to that obtained from a fluorecent microscope) to a droplet volume distribution [1, 2, 3]. The difference in distribution of the observed radii of circles that represent sections of droplets to the actual sphere radii arises from two reasons. The probability of a cross-section corresponding to the droplet radii is very low and the actual radii of the circles are less as viewed when the plane is not intersecting the center of the sphere. Secondly, in a polydisperse sample with a distribution of different sizes, the probability of a plane through the larger spheres are higher than for the smaller spheres, hence favoring the observation of the larger spheres. The one effect decreasing the observed droplet radii and the second effect increasing the droplet radii do counteract each other if the particle size distribution follows the Rayleigh distribution. The Rayleigh distribution is not very different to the expected log-normal distribution for an emulsion system, but with less of a tail for bigger droplets. For the purpose of this system, we are considering the Rayleigh distribution to be a valid approximation for the emulsion system, and only small differences in droplet radii and volume fraction can be expected from droplet radii observed in the micrographs. The distribution curves for the radii are shown in Figure S4. The measured samples were seen to be stable for 7 days [4]. Again, the constant droplet size with time suggests that the pea protein contributes to network formation, viscosity increase, and entrapment of the oil droplets that do not readily coalesce.

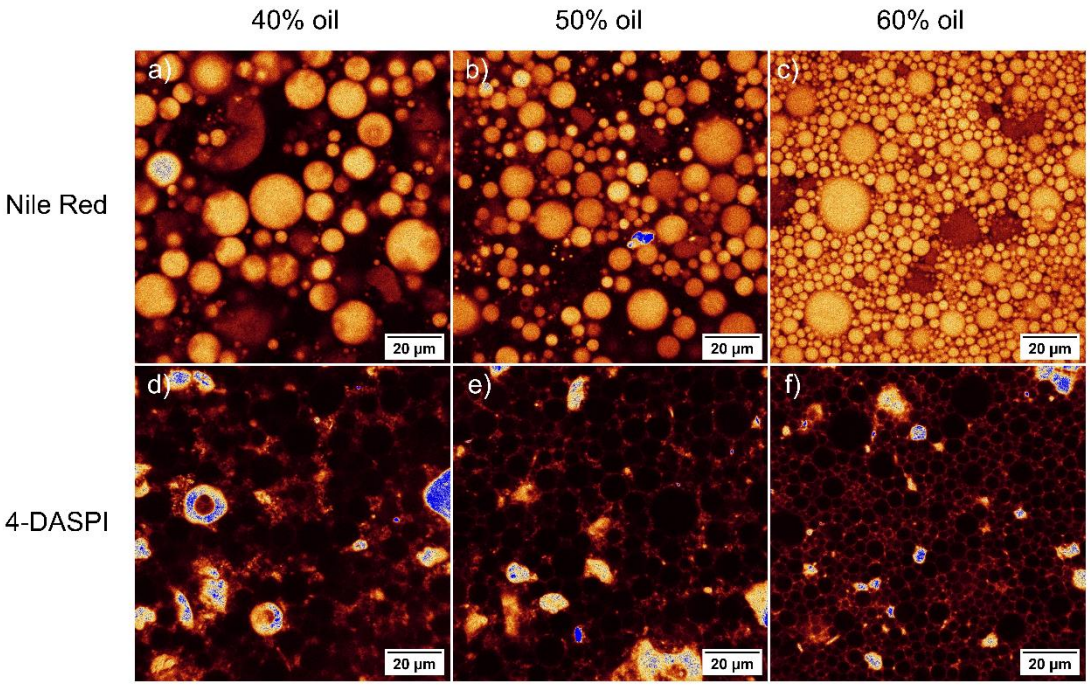

Figure S1. Confocal micrographs of emulsions stabilized with 7.5% w/v pea protein after storage at 19 °C for 7 days. The emulsions are stained with Nile Red (a, b, c) and 4-DASPI (d, e, f), respectively. The oil content is 40% v/v (a and d), 50% v/v (b and e) and 60% v/v (c and f) and the mean droplet radii are 3.5, 2.6 and 1.5 µm for the different oil fractions, respectively. The scale bars in the lower right corners of each image are 20 µm.

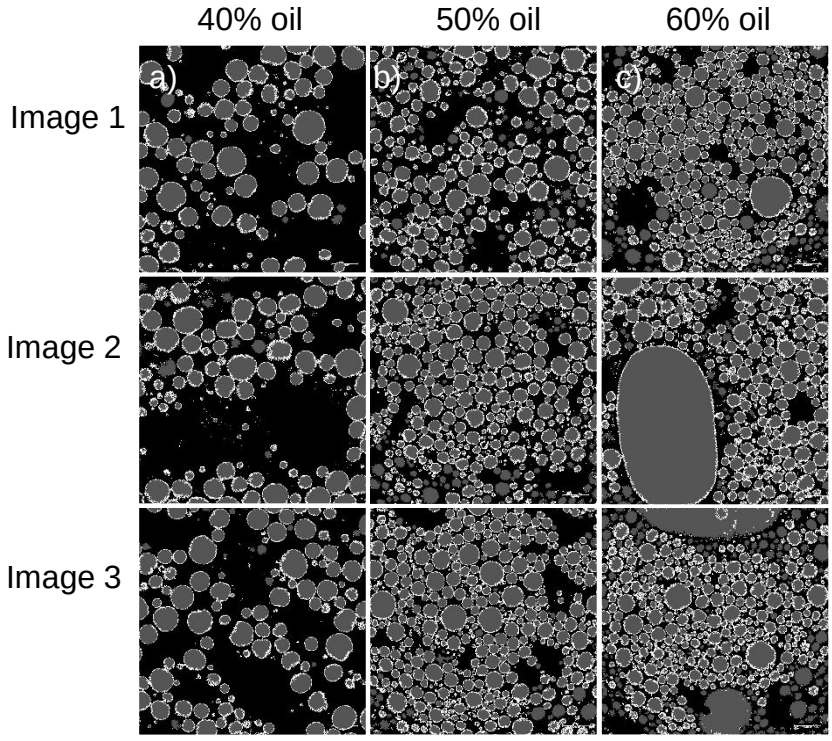

Figure S2. Analysis of the confocal micrographs of fresh emulsions stained with Nile Red. The emulsions are stabilized with 7.5% w/v pea protein. The oil content from left to right is 40, 50, 60% v/v and three images from each sample were analysed.

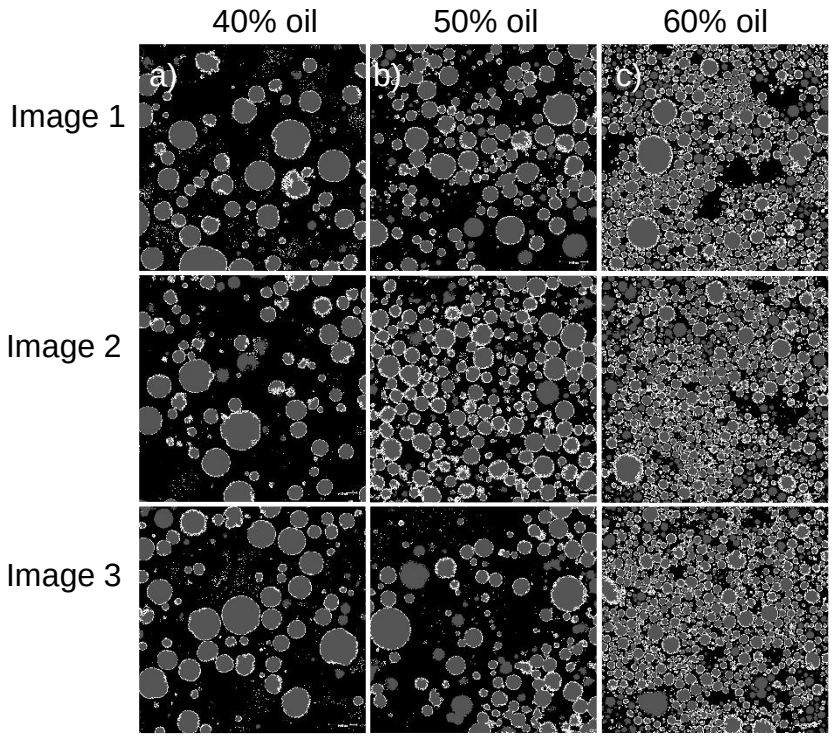

Figure S3. Analysis of the confocal micrographs of stable emulsions after 48 h of storage at 19°C stained with Nile Red. The emulsions are stabilized with 7.5% w/v pea protein. The oil content from left to right is 40, 50, 60% v/v and three images from each sample were analysed.

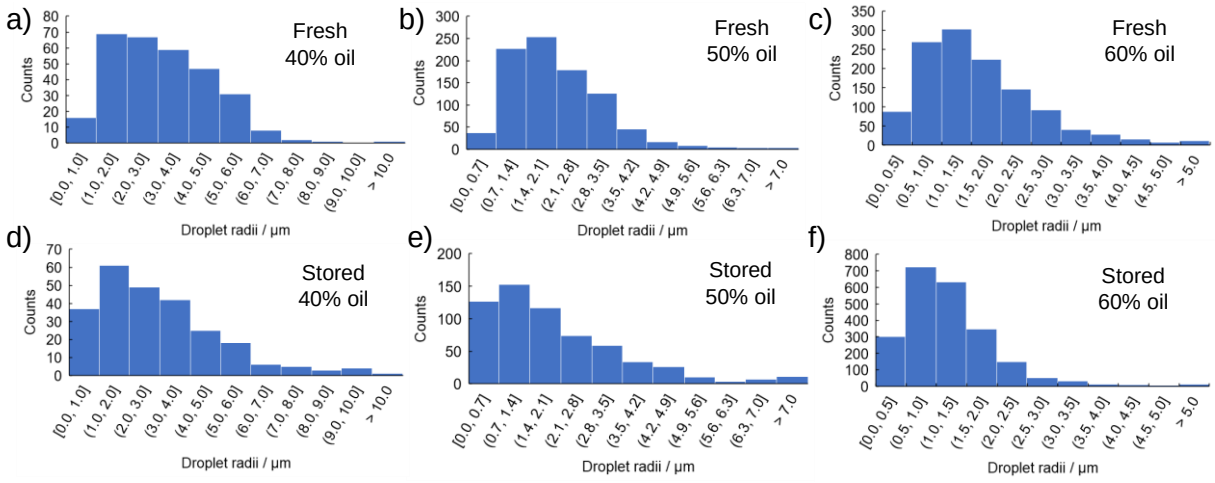

Figure S4. Size distribution curves from left to right for emulsions with 40, 50 & 60% v/v oil after preparation (top) and after storage for 7 days at 19°C (bottom).

2. Rheology of stable emulsions - compositions with 7.5% w/v protein

Creep measurements of shear rate under constant applied stresses are presented in Figure S5. The cut-off stress below which the emulsion stops flowing altogether is taken as the yield stress. The decrease in shear rate below a critical applied stress, likely indicates a thixotropic behaviour, confirming the observation from confocal microscopy of protein interactions in the bulk phase and opposing the idea of simple Pickering stabilization. Although a Pickering emulsion could also exhibit thixotropic behaviour due to the rearrangement of particles at the interface under applied stress [5], it seems likely that a network is forming in the bulk phase as we discuss in the main text. That is particular obvious from the observation of a yield stress also for the aqueous dispersion of 15% w/v pea protein in Figure 3 in the main text. It is apparent in Figure S5b that due to the complexity and the polydispersity of the systems, the absolute value for the shear stress should not be overanalysed, but rather simply noting that yield is observed. It is interesting instead to note the possibility of a time dependent yield stress or strain.

Sample	Yield stress (τ_y)	Consistency (κ)	Flow index (n)
60% oil, 7.5% protein	19.5	23.7	0.43
50% oil, 7.5% protein	0.02	0.80	0.75
40% oil, 7.5% protein	0.01	0.17	0.88
15% protein	0.33	0.80	0.74

Table S1. Parameters for the Herschel-Bulkley model fits (Equation 1), which are plotted in Figure 3 in the main text.

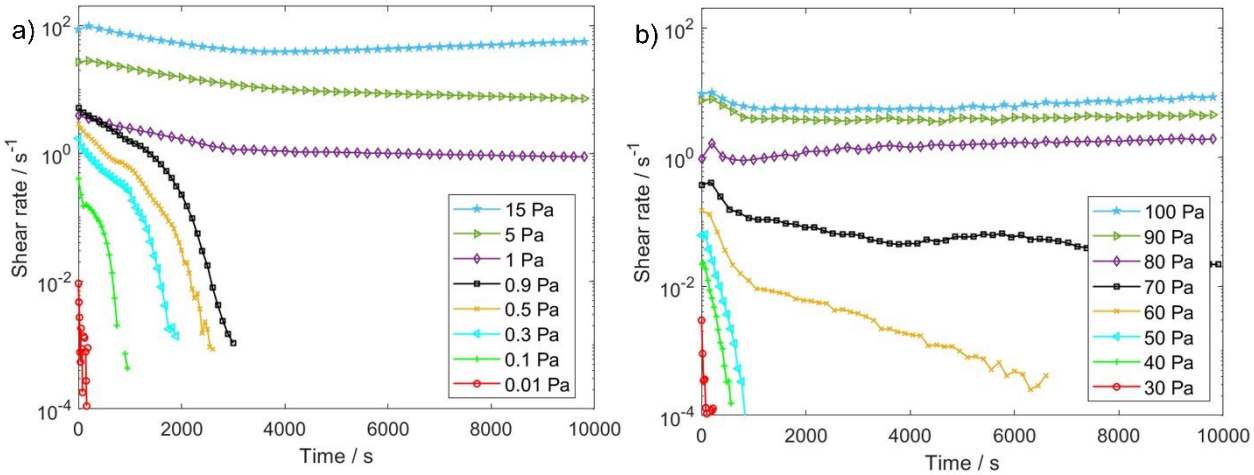

Figure S5. Shear rate response with time at various applied constant shear stresses for emulsions with 7.5% w/v pea protein. a) 40% v/v oil has a yield stress around 1 Pa. b) 60% v/v oil has a yield stress around 70 Pa.

3. Effects of evaporation

Small amounts (~1 mL) of pea protein dispersions in water (7.5 and 15% w/v) and emulsions (40, 50 and 60% v/v oil, and 7.5% w/v pea protein) were placed on petri dishes and left at 19°C. The weight of the samples was monitored as a function of time and the evaporation rates were approximated to a linear behavior for times less than 80 min. The evaporation rate was seen as significantly higher for the sample with 7.5% w/v pea protein than for the other samples. This difference is attributed to the emulsion systems and the 15% w/v dispersion having similar protein concentration in the aqueous phase. The rate of evaporation, as shown in Table S2, was also seen to decrease for higher oil content emulsions as would be expected. In a time of 5000 s corresponding to the rheological measurements in Figure 5, emulsion sample weights decrease with 13-16%. Assuming that the evaporation takes place in the aqueous phase, final oil concentrations increase by 8-9% (Table S2). The time effects on *G*' and *G*'' in Figure 5 are greater in the sample with initial oil concentration of 40% and less or equal in the sample with initial oil concentration of 50%. We observe that the strain amplitude sweeps look considerably different after rheological treatments. Taking this into account, there are significant drying affecting the final compositions, but the rheological effects are greater than what could be explained solely by drying effects.

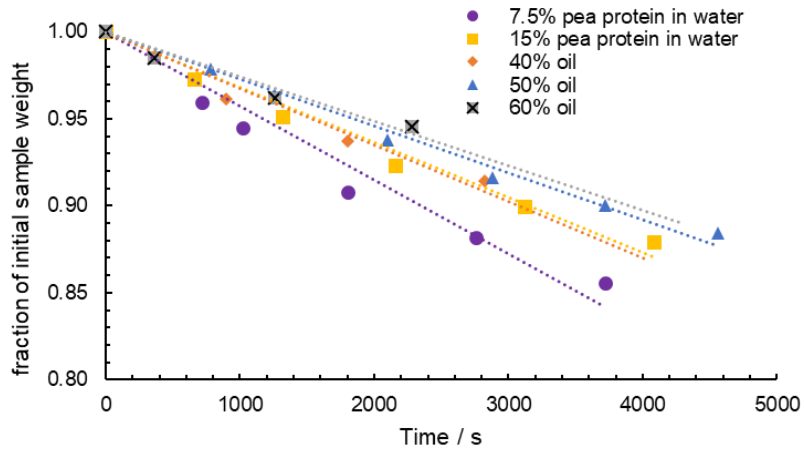

Figure S6. Evaporation rates for 7.5 and 15% w/v pea protein dispersions in water, and for emulsions with 40, 50 and 60% v/v oil stabilized with 7.5% w/v pea protein.

Oil concentration / % v/v	Total pea protein concentration / % w/v	Pea protein concentration in the aqueous phase / % w/v	Evaporation rate / 10^{-5} weight fraction s^{-1}	Oil concentration after 5000 s / % v/v
-	7.5	7.5	-4.3	-
-	15	15	-3.2	-
40	7.5	12.5	-3.2	48
50	7.5	15	-2.7	58
60	7.5	17.5	-2.6	69

Table S2. Evaporation rates for 7.5 and 15% w/v pea protein dispersions in water, and for emulsions with 40, 50 and 60% v/v oil stabilized with 7.5% w/v pea protein.

4. Confocal microscopy and density of emulsion compositions that separate

The different layers of the phase separated emulsions were studied. Confocal images of the aqueous middle phase are shown in Figure S7 (oil stained with Nile Red) and Figure S13 (protein stained with 4-DASPI). As can be seen from the micrographs in Figure S7, the oil concentration in this phase is very low with small fractions of impurities or oil droplets. Confocal images of the upper creaming phase are presented in Figure 6 and Figure S12. Further discussion will be focused on the upper creaming phase of the separated emulsions. The fraction of oil in this layer and the droplet size was estimated by droplet area coverage in the images as described in Section 1 and a summary of the results are shown in Table S3. Analysis of droplet sizes in Table 2 were made in the same way.

Measurements of the density were perfomed to confirm the oil, water, and pea protein fractions in the different layers. The results in Figure S14 shows an aqueous bottom phase with a density close to that of water. Additionally, the density of the top phase is around 0.96 g cm^{-3}, corresponding well with an oil fraction around 50% v/v and is approximately independent of the starting composition.

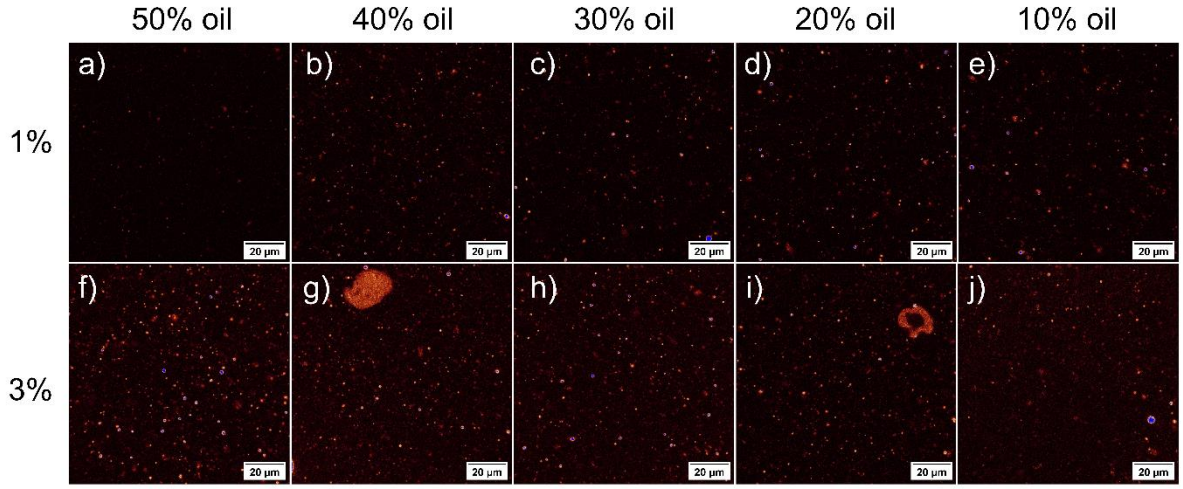

Figure S7. Confocal micrographs of the middle aqueous phase of separated emulsions after 48 h of storage at 19°C stained with Nile Red. The emulsions are stabilized with 1% (a-e) and 3% (f-j) w/v pea protein, respectively. The oil content of the original emulsions from left to right is 50, 40, 30, 20, 10% v/v. The scale bars in the lower right corners of each image are 20 µm.

Original oil concentration / % v/v	Original pea protein concentration / % w/v	Oil concentration in upper creaming phase / %	Mean droplet radius in upper creaming phase / µm
50	1	64	12 ± 2.2
40	1	60	10 ± 2.1
30	1	53	7.4 ± 1.9
20	1	54	8.9 ± 2.0
50	3	53	7.0 ± 1.9
40	3	54	6.6 ± 1.9
30	3	53	7.5 ± 2.0
20	3	38	5.5 ± 1.6
10	3	34	5.0 ± 1.5

Table S3. Composition and droplet size of the creaming layer of phase separated emulsions after storage for 48 h at 19°C as evaluated by confocal microscopy images. The uncertainty is one standard deviation.

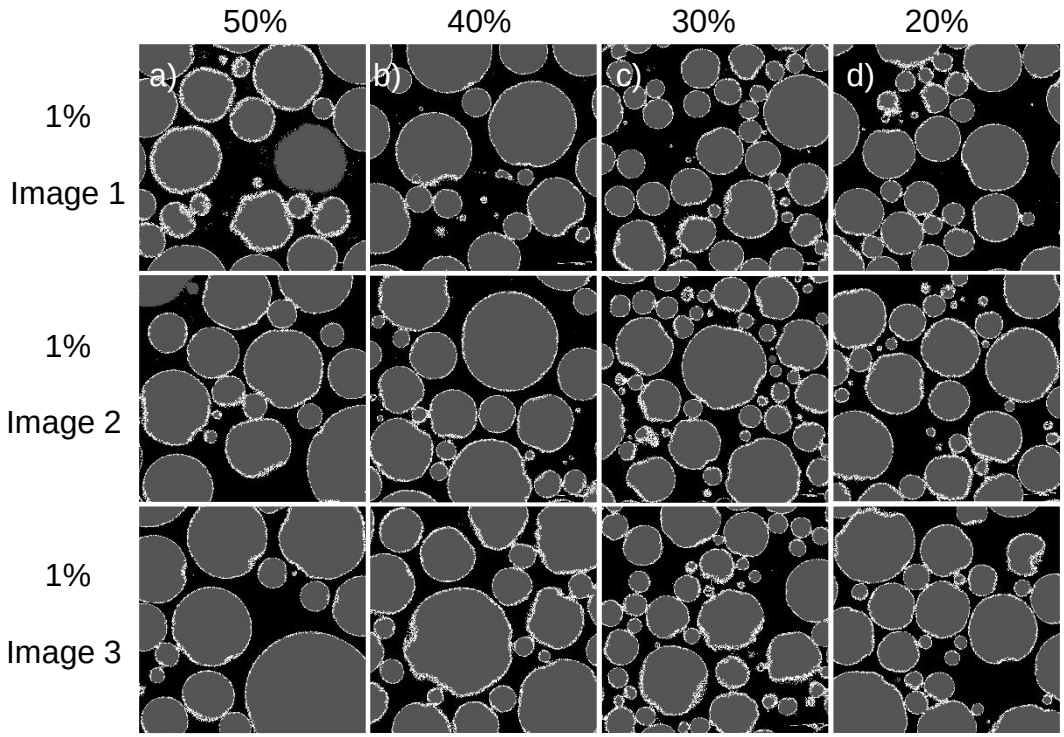

Figure S8. Analysis of the confocal micrographs of the top creaming phase of separated emulsions after 48 h of storage at 19°C stained with Nile Red. The emulsions are stabilized with 1% w/v pea protein. The oil content from left to right is 50, 40, 30, 20% v/v and three images from each sample were analysed. The sample with 10% oil did not produce enough creaming phase to be analysed in this way.

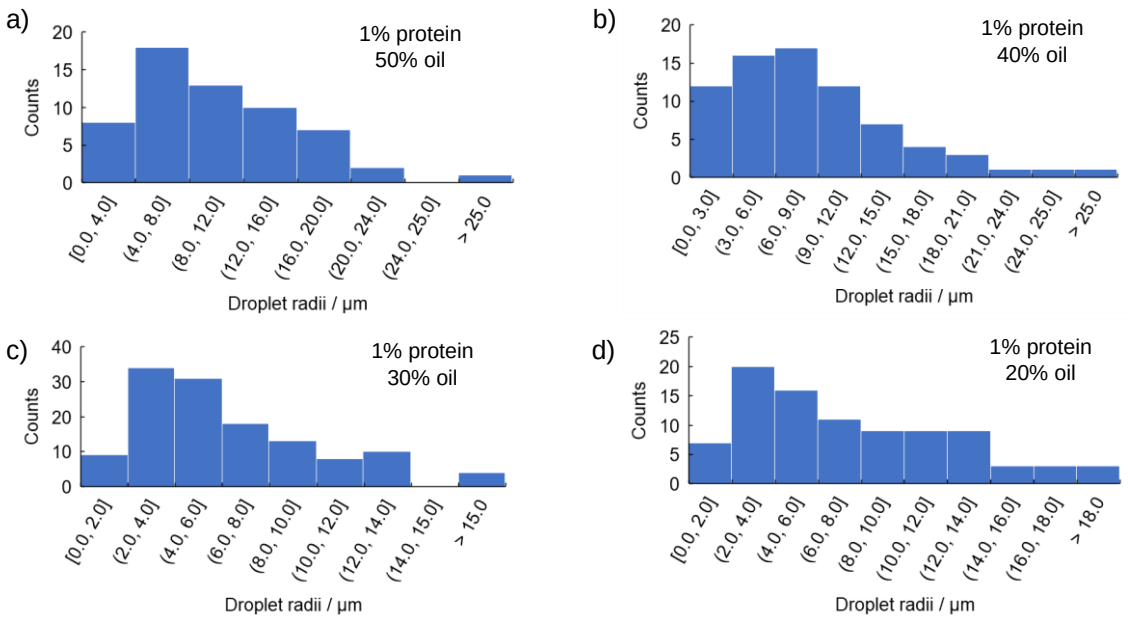

Figure S9. Size distribution curves for the top creaming phase of separated emulsions after 48 h of storage at 19°C. The emulsions are stabilized with 1% w/v pea protein. The initial oil content was a) 50%, b) 40%, c) 30% and d) 20% v/v oil.

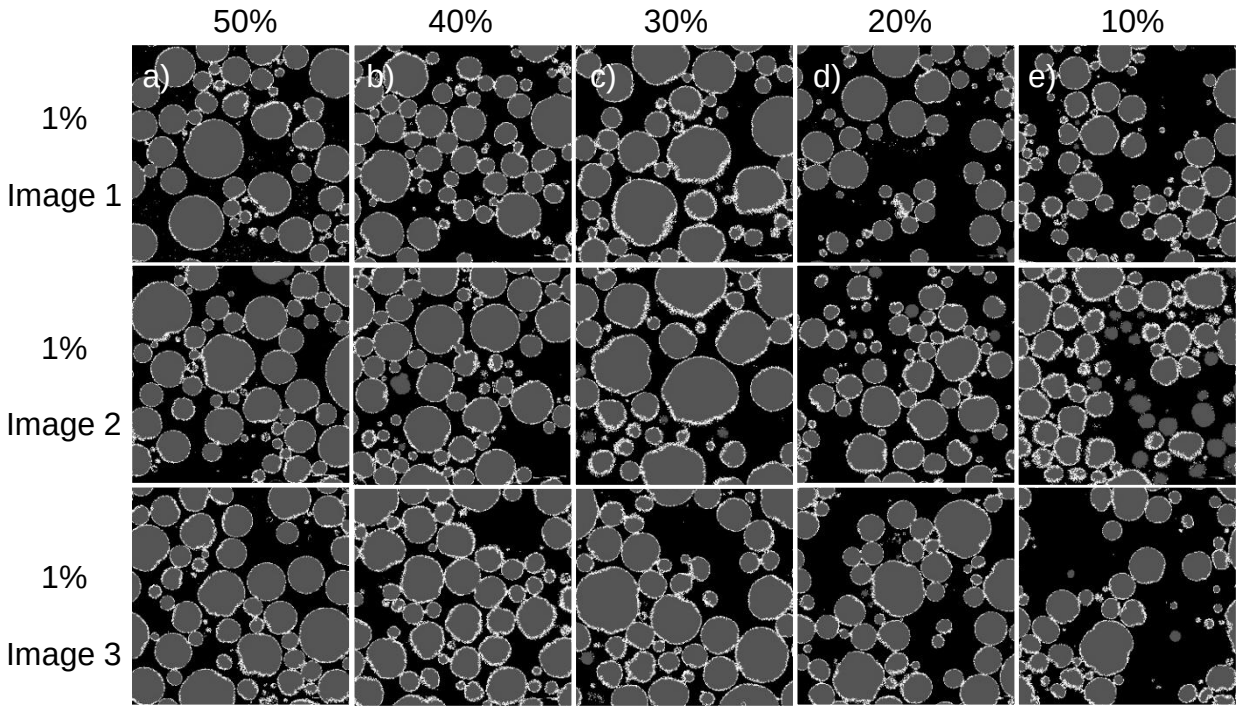

Figure S10. Analysis of the confocal micrographs of the top creaming phase of separated emulsions after 48 h of storage at 19°C stained with Nile Red. The emulsions are stabilized with 3% w/v pea protein. The oil content from left to right is 50, 40, 30, 20, 10% v/v and three images from each sample were analysed.

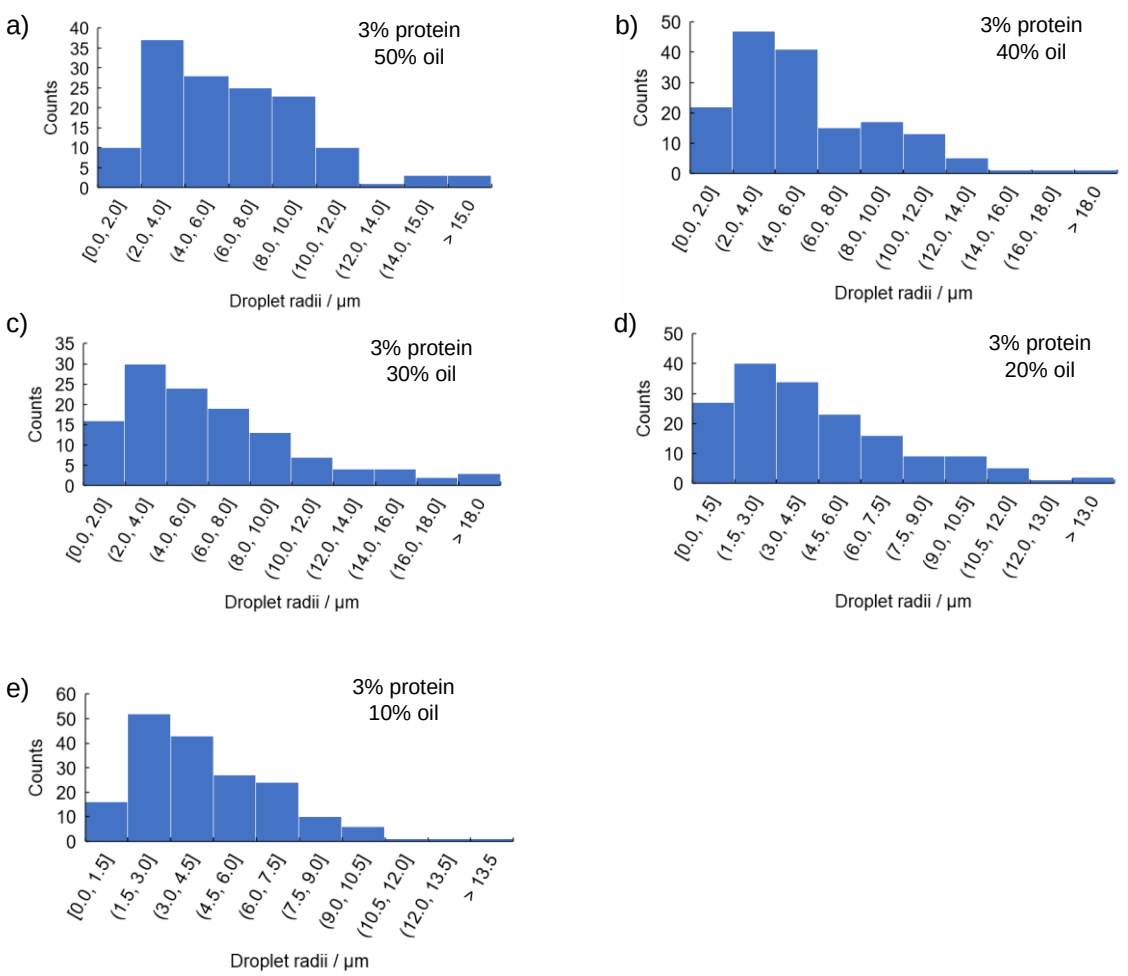

Figure S11. Size distribution curves for the top creaming phase of separated emulsions after 48 h of storage at 19°C. The emulsions are stabilized with 3% w/v pea protein. The initial oil content was a) 50%, b) 40%, c) 30%, d) 20% and e) 10% v/v oil.

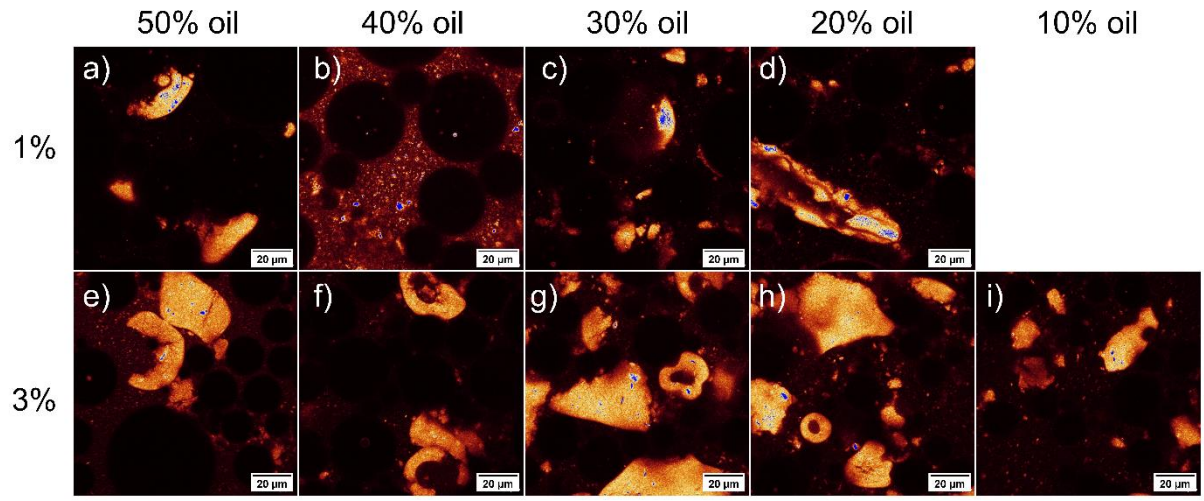

Figure S12. Confocal micrographs of the upper creaming phase of separated emulsions after 48 h of storage at 19°C, stained with 4-DASPI. The emulsions are stabilized with 1% (a-d) and 3% (e-i) w/v pea protein, respectively. The oil content for the original emulsions from left to right is 50, 40, 30, 20, 10% v/v. The sample with 10% oil and 1% pea protein did not produce enough creaming phase to be analysed in this way. The scale bars in the lower right corners of each image are 20 µm.

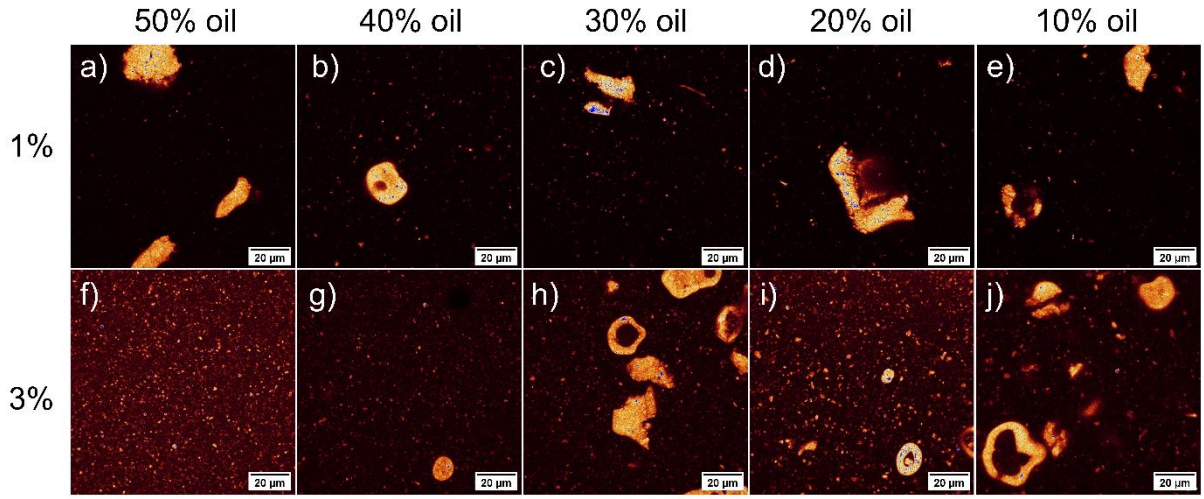

Figure S13. Confocal micrographs of the middle aqueous phase of separated emulsions after 48 h of storage at 19°C stained with 4-DASPI. The emulsions are stabilized with 1% (a-e) and 3% (f-j) w/v pea protein, respectively. The oil content for the original emulsions from left to right is 50, 40, 30, 20, 10% v/v. The scale bars in the lower right corners of each image are 20 µm.

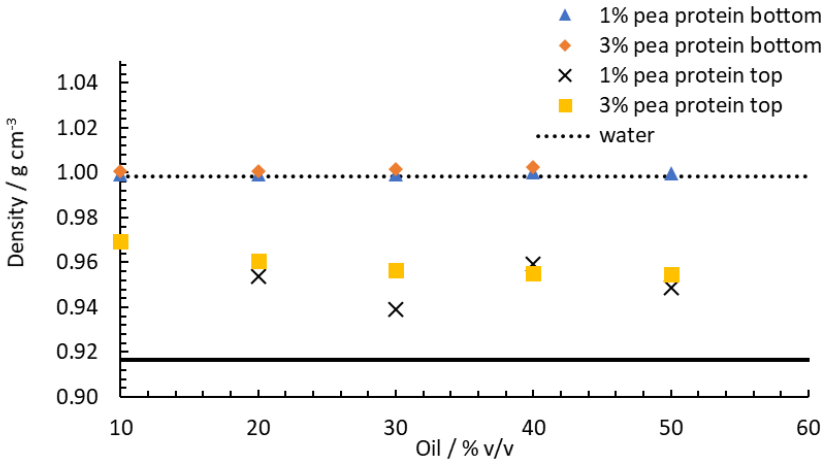

Figure S14. Density of the aqueous bottom phases (blue triangles and red diamonds) and the creaming top phases (black crosses and yellow squares) in phase separated samples with starting compositions 10-50% v/v oil and with 1 and 3% w/v pea protein, respectively. The density is plotted against the original oil fraction in the samples.

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