Evaluation of Short-Term and Long-Term Stability of Emulsions by Centrifugation and NMR*

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Abstract. The effect of storage time on the coalescence stability and drop
size distribution of egg yolk (EY) and whey protein concentrate (WPC) sta-
bilized emulsions is studied. The emulsion stability is evaluated by centrifu-
gation, whereas the drop size distribution is measured by means of NMR and
optical microscopy. The experimental results show that there is no general re-
lation between the emulsion stability and the changes in the mean drop diam-
eter upon shelf-storage of protein emulsions. On the other hand, it is shown
that the higher short-term stability, measured by centrifugation immediately af-
ter emulsion preparation, corresponds to higher long-term stability (after their
self-storage up to 60 days) for emulsions stabilized by the same type of emulsi-
fier. In this way, we are able to obtain information for the long-term stability of
emulsions in a relatively short period of time.

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1 Introduction

One of the methods for evaluation of emulsion stability is to monitor the changes
in the mean drop size for a certain period of storage time [1-3]. It is often con-
sidered that an increase of drop size with time is a clear indication for emulsion
destabilization [1-3]. Such a relation is quite understandable (at a first glance)
for at least two reasons: (1) The increase of drop size reflects an ongoing co-
alescence process inside the emulsion; (2) As shown in previous studies [4-6],
the stability of emulsions containing larger drops is lower at equivalent other

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*This work is dedicated to Professor Alexander Derzhanski , DSci, Corresponding Member of
the Bulgarian Academy of Sciences on the occasion of his 70th anniversary.

conditions. On the other hand, we have found that the coalescence stability of BLG emulsions significantly decreases after one day of storage, as compared to the stability of freshly prepared emulsions (aging effect) [6], while the drop size distribution remains practically unchanged.

In the present paper we study protein stabilized emulsions with the aim to clarify what is the relation between the variations in the drop size and the stability of these emulsions in the course of their long-term storage. On the basis of the obtained results we formulate a "rapid" procedure for evaluation of the long-term stability of emulsions.

2 Materials and methods

2.1 Materials

Whey protein concentrate (WPC; trade name AMP 8000; product of Proliant) and whole liquid Egg Yolk (EY) were used for emulsion preparation. Soybean oil (SBO), purified by passing it through a glass column filled with Florisil adsorbent, was used as an oil phase. The aqueous solutions were prepared with deionized water, purified by a Milli-Q system (Millipore). Along with the main emulsifier, the solutions contained NaCl (Merck, analytical grade, heated for 5 hours at 450°C) and NaN_3 (Riedel-de Haën).

2.2 Emulsion Preparation

(A) Egg Yolk stabilized emulsions. Oil-in water emulsions (78 vol.% SBO) were prepared by using a two-step procedure. A coarse emulsion was prepared by using a kitchen mixer. First, 115 mL of the yolk solution was stirred for 1 min and then 405 mL oil (78 vol.%) was added at a constant rate for 15 min. The mixing continued for additional 10 min after introducing the last portion of oil. Then 10 mL of 12% acetic acid was added to the coarse emulsion and the latter was homogenized by the mixer for another 10 min.

The second homogenization step was accomplished by passing this premix through the slit of a narrow-gap homogenizer. The mixing head of this homogenizer has a processing element, which contains a narrow slit with gap width of 395 μm. We applied a pressure of 3 bar at the inlet chamber of the cavitation mixer. The flow rate was 0.08 L/s under this pressure. The duration of the emulsification during this second stage was fixed at 10 min, which corresponded to 100 cycles (passages) of the emulsion. A closed loop (circulation of the emulsion) was used for these multiple passages.

(B) WPC stabilized emulsions. Oil-in water emulsions (28 vol.% SBO) were prepared by using a two-step formation procedure. Initially, a premix was prepared with 500 mL WPC solution and 200 mL SBO, which were homogenized

by hand. The final homogenization (second step) was accomplished by a narrow-gap homogenizer at a pressure of 2 bar. The flow rate was 0.13 L/s in this system. The duration of the emulsification was fixed at 10 min.

The freshly prepared emulsions were transferred in centrifugal test-tubes, capped, and stored in a dark box at room temperature. Two series of samples were prepared for the emulsions stabilized by 12 or 19 wt.% EY in the aqueous phase — in one of the series, the emulsions were stored in plastic (polypropylene tubes), whereas the emulsions were stored in glass tubes in the second series. No significant effect of the tube material on the evolution of the drop size and emulsion stability was noticed. The WPC emulsions were stored only in polypropylene tubes.

2.3 Determination of Drop Size Distribution

(A) NMR measurements were used to determine the mean drop size and the drop size distribution in the studied emulsions. The NMR measurements were performed on Bruker Minispec mq20 NMR apparatus with the Bruker application program "oil droplets.app".

This application program uses the model of restricted diffusion of oil molecules in spherical droplets of uniform size and the attenuation of the NMR echo signal in this configuration is given by the formula [7]:

$$
\ln[R] = -2\gamma^2 g^2 \sum \frac{1}{\alpha_m^2(\alpha_m^2 a^2 - 2)}
$$
$$
\times \left(\frac{2\delta}{\alpha_m^2 D} - \frac{2 + \exp(-\alpha_m^2 D(\Delta - \delta)) - 2\exp(-\alpha_m^2 D\delta)}{(\alpha_m^2 D)^2} - \frac{2\exp(-\alpha_m^2 D\Delta) - \exp(-\alpha_m^2 D(\Delta + \delta))}{(\alpha_m^2 D)^2} \right) \quad (1)
$$

where a is the drop radius, D is the self-diffusion coefficient of the oil molecules in bulk oil, and α_m is the mth positive root of the equation:

$$
\frac{1}{\alpha a} J_{3/2}(\alpha a) = J_{5/2}(\alpha a), \quad (2)
$$

In the case of distribution of the drop sizes, the measured echo attenuation ratio R_{obs} can be presented by the following equation [8]:

$$
R_{obs} = \frac{\int_0^\infty a^3 P(a) R(\Delta, \delta, g, a)\, da}{\int_0^\infty a^3 P(a)\, da} \quad (3)
$$

where $R(\Delta, \delta, G, a)$ is given by Eq. (1) and $P(a)$ is the drop size distribution function. In the "oil droplets.app" program, a log-normal distribution is assumed

for $P(a)$

$$P(a) = \frac{1}{2a\sigma\sqrt{2\pi}} \exp\left[-\frac{(\ln 2a - \ln d_{33})^2}{2\sigma^2}\right] \tag{4}$$

In this equation, d_{33} is the volume weighted mean drop diameter ($d = 2a$), σ is the standard deviation of the logarithm of the drop diameter (σ is a dimensionless quantity).

The following parameters were used in our experiments: $T = 23.5$ °C, $\Delta = 210$ ms, $\delta_{max} = 2.5$ ms, number of test points $n = 9$, magnetic gradient $G = 2.0$ T/m, diffusion coefficient of SBO $D_{oil} = 1.15 \times 10^{-11}$ m²/s (measured in an independent experiment).

(B) Optical microscopy. The mean drop size and the drop size distribution were determined by optical microscopy, as well. The specimens prepared for optical microscopy were taken from the same samples, which were transferred into the NMR tubes. The microscopy samples were diluted in 20 mM SDS solution to prevent the drop flocculation and coalescence. The oil drops were observed in transmitted light with a microscope Axioplan (Zeiss, Germany), equipped with objective Epiplan, ×50, and connected to CCD camera (Sony) and VCR (Samsung SV-4000). The diameters of the recorded oil drops were afterward measured (one by one) by using a home-made, image analysis software, operating with Targa+ graphic board (Truevision, USA).

2.4 Evaluation of the emulsion stability by centrifugation

After a certain period of shelf-storage, the emulsions were centrifuged at 20°C in 3K15 centrifuge (Sigma Laborzentrifugen, Germany). The emulsion stability was characterized by the critical osmotic pressure, P_{osm}^{cr}, at which an oil layer is released at the top of the emulsion cream in the centrifuge tube [4,6]. P_{osm}^{cr} is calculated from the experimental data, under the assumption that the centrifugal acceleration is homogeneous along the cream, by using the following equation [4,6]:

$$P_{osm}^{cr} = \Delta\rho g_k(V_{oil} - V_{rel})/A_{TT} \tag{5}$$

where $\Delta\rho$ is the difference between the mass densities of the oil and the aqueous phases; g_k is the centrifugal acceleration; V_{oil} is the total volume of oil used for preparation of the emulsion; V_{rel} is the volume of released oil at the end of centrifugation; and A_{TT} is the cross-sectional area of the centrifuge test tube (see Figure 1).

2.5 Emulsion stability under shear

The studied emulsion was transferred into a glass container with a cylindrical shape (inner diameter 2.8 cm). Afterwards, a stainless steal cylinder with an
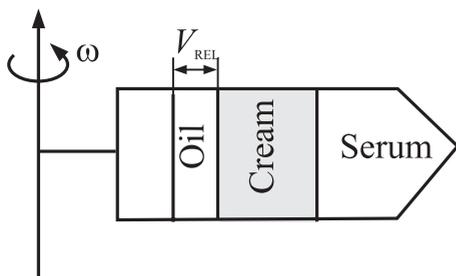
Figure 1. Schematic presentation of an emulsion in a centrifugal tube. V_{rel} is the volume of released oil at the end of the centrifugation.
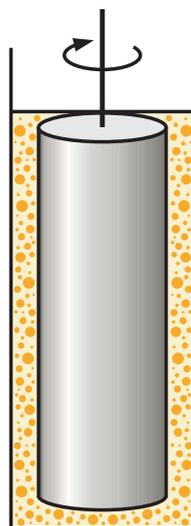

Figure 2. Schematic presentation of an emulsion subjected to shear deformation.

outer diameter of 1.9 cm was co-axially introduced into this emulsion and rotated at a constant rotation speed of 250 rpm (see Figure 2). In this way, the emulsions were sheared for 15 min at a shear rate of 55 s^{-1}. The drop size distribution was determined by NMR and optical microscopy before and after shearing of the emulsions for comparison.

3 Results and Discussion

3.1 EY stabilized emulsions

This series of experiments was performed with 78 vol.% SBO emulsions, stabilized by two different EY concentrations — 12 and 19 wt.% EY (the solutions contained also 1 M NaCl and 0.01 wt.% NaN$_3$; pH was adjusted by acetic acid to 4.0).

The emulsion stability against coalescence upon shelf-storage was investigated as follows: A series of centrifugal tubes (around 15 tubes for one batch of emulsion) was filled with equal amounts of freshly prepared emulsion. Afterwards, these samples were stored undisturbed at room temperature for different periods of time — from 1 day up to 60 days. We call "the storage period" the time between the preparation of an emulsion and its investigation. The samples for drop size distribution measurements by NMR and optical microscopy were
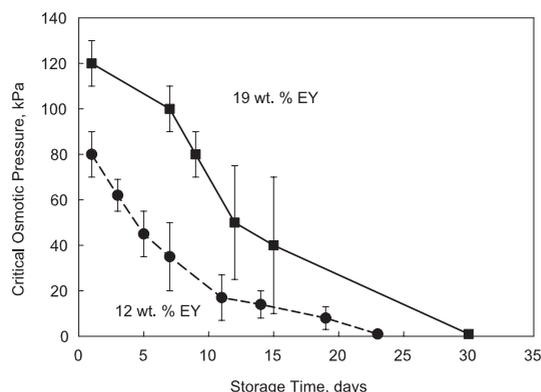
Figure 3. Critical osmotic pressure, P_{osm}^{cr}, as a function of storage time for oil-in-water emulsions stabilized by 12 wt.% EY (full cycles) or 19 wt.% EY (full squares). Along with EY, all solutions contain 1 M NaCl, 0.01 wt.% NaN$_3$ at pH = 4.0.

taken from the middle part of the emulsion at the end of the storage period, so that the dependence of drop size distribution on storage time was determined. Other samples from the same batch emulsion were centrifuged to determine the critical osmotic pressure for coalescence, P_{osm}^{cr}, so that the dependence of P_{osm}^{cr} on storage time was also evaluated.

In Figure 3 we present the results for P_{osm}^{cr} as a function of storage time for emulsions stabilized by 12 wt.% and by 19 wt.% EY. The points represent the mean values of P_{osm}^{cr} measured with 3 to 5 independently prepared emulsions.

The results presented on Figure 3 show that the emulsion stability decreased almost linearly with storage time for the lower EY concentration (12 wt.%). It should be noted that we observed oil lenses on top of the emulsion even without centrifugation at the end of the storage period (about 20 days of storage) for this EY concentration. A bulk oil layer was released on top of these emulsions after 25 days of storage. The volume of the released oil was around 2 mL, which was ≈ 10% of the oil, used for preparation of each sample. The amount of released oil increased with storage time. We noticed also that a protein serum (aqueous phase without oil drops) separated below these emulsions after 40 days of storage. In parallel, we observed the formation of large cracks, filled with serum, in the remaining emulsion, which is an indication that a process of emulsion compaction (syneresis) takes place.

Similar dependence of emulsion stability on storage time was obtained for emulsions stabilized by 19 wt.% EY (see Figure 3), but several differences were noticed: The time required for spontaneous formation of oil lenses on top of these emulsions, without centrifugation, was about 1.5 times longer (about 30 days). No formation of cracks and separation of aqueous phase were observed for stor-
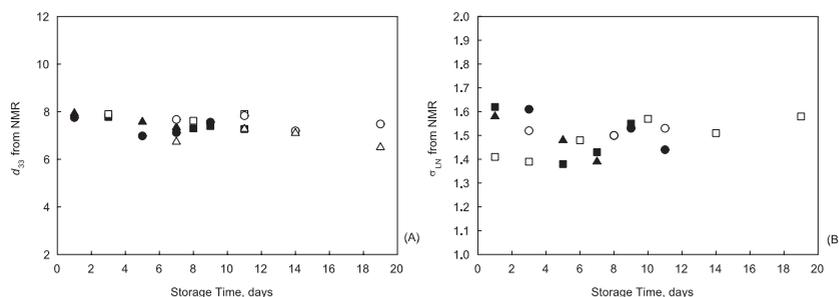
Figure 4. Time dependence of (A) mean volume averaged diameter, d_{33}, and (B) dimensionless width of drop size distribution, σ_{LN}, as measured by NMR, for emulsions stabilized by 12 wt. EY (78 vol.% SBO, 1M NaCl, 0.01 wt.% NaN$_3$, pH = 4.0). The different symbols represent data for different batch emulsions.

age time up to 2 months. The experimental results for the emulsion stability after 10 days of storage were more scattered for this EY concentration.

The comparison of the critical osmotic pressure for destabilization of the two emulsions containing EY (see Figure 3) showed that the emulsion with 19 wt.% EY had higher stability than those containing 12 wt.% EY during the entire storage period. In other words, the higher short-term stability (measured immediately after emulsion preparation) corresponded to higher long-term stability (up to 30 days) for EY emulsions.

As discussed in the introduction, one of the reasons for the emulsion destabilization during storage could be an actual increase of drop size due to drop-drop coalescence [4]. That is why, in parallel with the evaluation of emulsion stability, we measured the drop size distribution in the stored emulsions. The obtained NMR results for the volume-averaged mean diameter, d_{33}, and for the width of the size distribution, σ_{LN}, are presented in Figure 4 as functions of the storage time for six different batch emulsions, stabilized by 12 wt.% EY. For the samples, taken from one batch emulsion, the values of d_{33} varied by no more than ± 0.3 μm ($\pm 5\%$) during the entire storage period. The obtained results for σ_{LN}, as a function of storage time, were more scattered ($\pm 7\%$), but no clear tendency for increase or decrease of σ_{LN} was detected. Similar dependence of d_{33} and σ_{LN} on storage time was obtained with emulsions stabilized with 19 wt.% of EY.

The drop-size distributions at the beginning (1 day of storage) and at the end of the storage period (20 days of storage for 12 wt.% EY and 30 days for 19 wt.% EY) were measured by optical microscopy as well, because the NMR method fails to detect oil drops of diameter larger than ca. 25 μm. The respective histograms for 12 wt.% EY emulsions are compared in Figure 5 — it is seen that no significant change in drop-size distribution for these emulsions takes place during storage (the same result was obtained for 19 wt.% EY). We can conclude
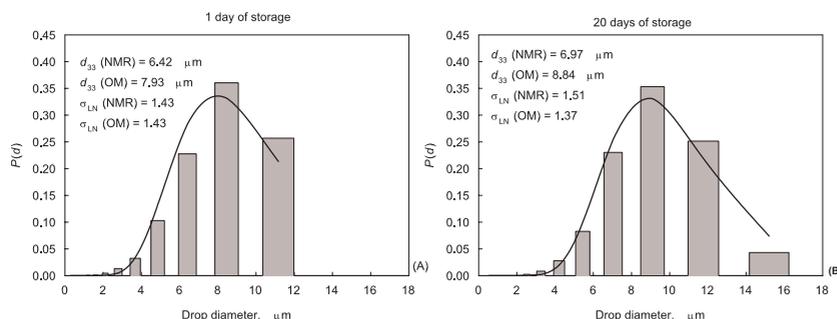
Figure 5. Histograms of the volume-weighted drop-size distribution after: (A) 1 day of storage and (B) 20 days of storage for soybean oil-in-water emulsion, stabilized by 12 wt.% EY (1 M NaCl, 0.01 wt.% NaN$_3$, pH = 4.0).

from these measurements that there are no significant changes in drop-size distributions with storage time for EY-stabilized emulsions.

To define the place where the emulsion destabilization predominantly occurs, we performed centrifugation of "spoiled" 19 wt.% EY emulsion (with released oil after 32 days of storage), after removing the released oil from the top of the emulsion. The oil layer was gently sucked together with the upper part of the cream, in which large oil lenses were observed by naked eye. The remaining middle part of the emulsion was afterward used for determination of emulsion stability by means of centrifugation. The measured P_{OSM}^{CR} was about 40 kPa, which corresponds to the stability of these emulsions after 15 days of storage. This result clearly indicates that the destabilization of EY emulsions during the storage period starts from the upper part of the emulsion cream, which is in contact with the air. It should be mentioned that the measured drop-size distribution for sample taken from the middle part of the cream was still the same as in the initial emulsion. The observation that the emulsion stability for the middle part of the cream was not as high as that of the initial emulsion, indicated that the changes in the adsorption layers, which led to the loss of stability, occurred in the entire emulsion column, but the largest changes are at the top, which is in contact with air.

The drop-size histograms before and after shearing, for 19 wt.% EY emulsion stored for one day, are compared in Figure 6. The comparison does not show significant changes in drop-size distribution as a result of shear. This result indicates that the applied shear stress is not sufficient to provoke drop-drop coalescence in this relatively fresh emulsion. Similar results were obtained with emulsions stored up to 15 days at both studied EY concentrations. However, significant difference between the drop-size histograms before and after shearing was obtained with emulsions stored 20 days, which were stabilized by 19 wt.% EY (see Figure 7). As one can see, the drop-size distribution after shearing ex-

Before shearing

d_{33} (NMR) = 4.74 μm
d_{33} (OM) = 5.61 μm
σ_{LN} (NMR) = 1.38
σ_{LN} (OM) = 1.44

After shearing

d_{33} (NMR) = 4.78 μm
d_{33} (OM) = 5.59 μm
σ_{LN} (NMR) = 1.44
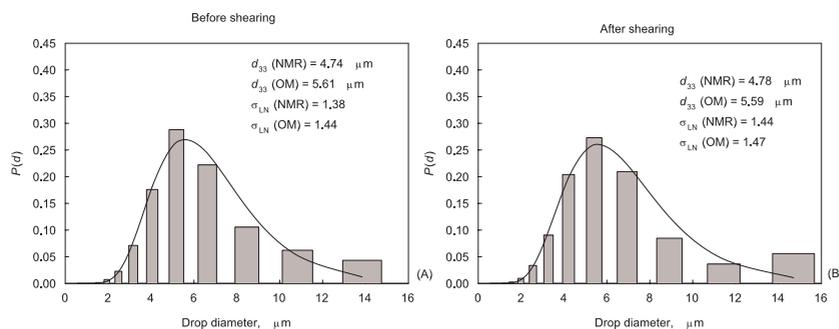σ_{LN} (OM) = 1.47

Figure 6. Histograms of the volume-weighted drop-size distribution of 12 days-stored emulsions stabilized by 19 wt.% EY (A) before and (B) after shearing.

hibited two peaks: the first peak closely resembled the size distribution in the initial emulsion, while the second peak corresponded to much larger drops, each of them formed by coalescence of many drops from the initial emulsion. No separation of free oil was observed after shear.

To define better where the emulsion destabilization took place upon shear, the following experiment was performed: The upper part and the lower part of an emulsion, stabilized by 12 wt.% EY and stored 18 days, were subjected to shear and the drop-size distributions before and after this treatment were measured and compared. The respective histograms are presented in Figure 8. There is no significant change in drop-size distribution between the upper and lower parts of the emulsion before shearing. However, formation of drops with larger diameters was observed for the upper part of the cream after shearing. On the contrary, no change in drop-size distribution was observed for the lower part of the cream after shearing. This result clearly show that the destabilization started in the upper part of the emulsion cream, which is in contact with air.

Before shearing

d_{33} (NMR) = 4.50 μm
d_{33} (OM) = 5.23 μm
σ_{LN} (NMR) = 1.38
σ_{LN} (OM) = 1.44

After shearing

d_{33} (NMR) = 5.00 μm
d_{33} (OM) = 9.11 μm
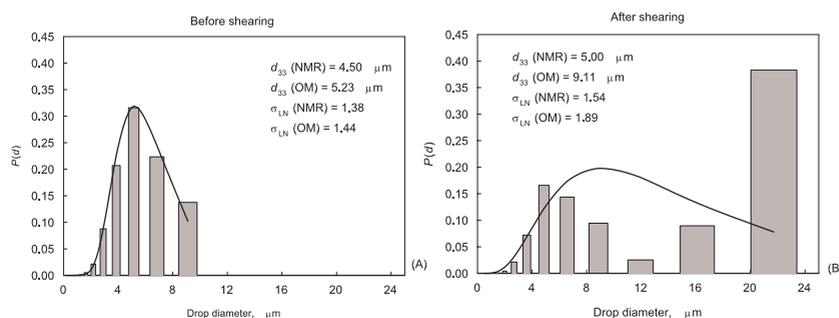σ_{LN} (NMR) = 1.54
σ_{LN} (OM) = 1.89

Figure 7. Histograms of the volume-weighted drop-size distribution of 20 days-stored emulsions stabilized by 19 wt.% EY (A) before and (B) after shearing.
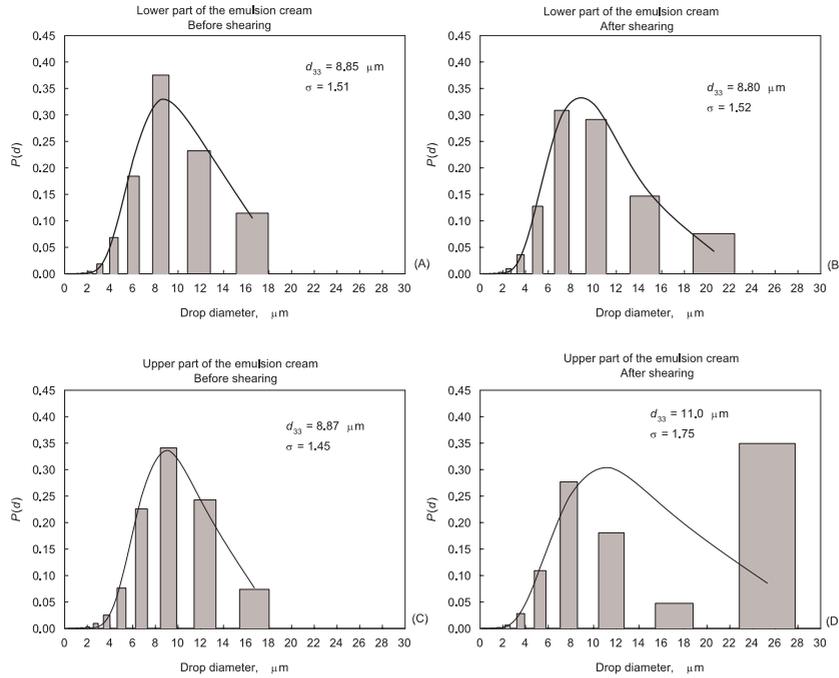
Figure 8. Histograms of the volume-weighted drop-size distribution for 15 days-stored emulsion stabilized by 12 wt.% EY from the (A) lower part of the emulsion cream before shearing; (B) lower part of the emulsion cream after shearing (C) upper part of the emulsion cream before and (D) after shearing.

Let us discuss the possible explanation of the observed stability decrease for EY- emulsions upon shelf-storage. We did not detect any tendency for increase of d_{33} and σ_{LN} (see Figure 4) at storage times up to 20 days for the studied systems (12 and 19 wt.% EY). Therefore, the decreased emulsion stability upon shelf-storage (aging effect) and after shear could not be explained by changes in drop-size. These results mean that the observed stability loss is due primarily to changes in the structure of the adsorption layer, which makes it less efficient as emulsion stabilizer.

One possible explanation of the aging effect is the formation of intermolecular bonds between the adsorbed protein molecules on the drop surface. Similar effect was observed with BLG-stabilized emulsion [6]. The explanation which was given for BLG emulsions was that strong intermolecular bonds are formed with time between the adsorbed protein molecules, which leads to a transformation of the adsorption layer into a fragile protein shell, which easily ruptures upon surface expansion. As explained in [4], the formation of a film in the point of contact between two neighboring drops (or its expansion in area) is accom-

panied with an inevitable extension of drop surfaces. If the adsorption layer is fragile, then bare (deprived of protein) oil-water spots could appear on the film surfaces, leading to film destabilization and drop-drop coalescence.

Indeed, Mine and Begougnoux [9] observed a gradual polymerization of the lipoprotein complexes at oil-water interfaces for mayonnaise-type emulsions stabilized by EY, as a result of strong hydrophobic interactions. We can speculate that this polymerization leads to similar transformation of the adsorbed lipoprotein layer into a fragile shell and to a subsequent decrease of emulsion stability upon storage, similarly to the case of BLG emulsions.

The observation of cracks formed throughout the emulsion column, after 40 days of storage, indicates a compaction of the emulsion column with an accompanying enhancement of drop deformation. Therefore, one could speculate that this compaction of the emulsions leads to an actual expansion of the drop surface and a possible rupture of the lipoprotein adsorption layers, which stabilize the films between the drops (with a subsequent drop-drop coalescence).

3.2   WPC stabilized emulsions

Similar series of experiments was performed with soybean oil-in-water emulsions (28 vol.%) stabilized by two different concentrations of WPC, 0.04 and 0.1 wt.% (the solutions contained also 0.15 M NaCl, 0.01 wt.% NaN_3, natural pH). The main difference between WPC and EY emulsions, except the emulsifier, was the oil volume fraction, which was 28 vol.% in WPC emulsions and 78 vol.% in EY emulsions. The lower volume fraction of WPC emulsion leads to a separation of a serum at the bottom of the centrifugal tube after several hours of shelf-storage. That is why, this serum was removed by a syringe before transferring the emulsion sample into the NMR tube. Samples for drop-size distribution by NMR and optical microscopy were taken from the middle part of the cream.

The obtained results for P_{osm}^{cr}, as a function of storage time, for the two studied concentrations of WPC are presented in Figure 9. We observed a 4-fold increase of emulsion stability with storage time for the emulsions containing 0.04 wt.% WPC, whereas P_{osm}^{cr} was almost constant during the entire storage period for emulsions stabilized by 0.1 wt.% WPC. The obtained values of P_{osm}^{cr}, for emulsions stabilized by 0.1 wt.% WPC, are higher than those obtained at 0.04 wt.% WPC during the entire period, which shows that the higher short-term stability (after 1 day of storage) corresponds to higher long-term stability, as well.

In parallel, the drop-size distributions were also measured. Histograms of the drop-size distribution, obtained by optical microscopy, for emulsions stabilized by 0.04 wt.%, at two different storage times, are presented in Figure 10. The values of d_{33} increased by about 25 % after 42 days of storage. This was mainly due to the appearance of oil drops with diameter larger than 30 μm, which were
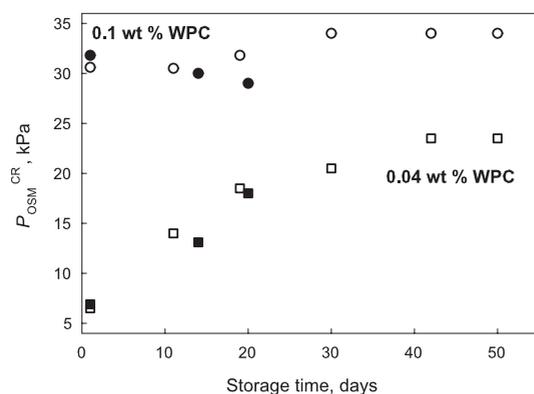
Figure 9. Critical osmotic pressure, P_{osm}^{cr}, as a function of storage time for oil-in-water emulsions stabilized by 0.04 wt.% (squares) or 0.1 wt.% WPC (cycles). Along with WPC, all solutions contain 0.15 M NaCl, 0.01 wt.% NaN$_3$ at natural pH.
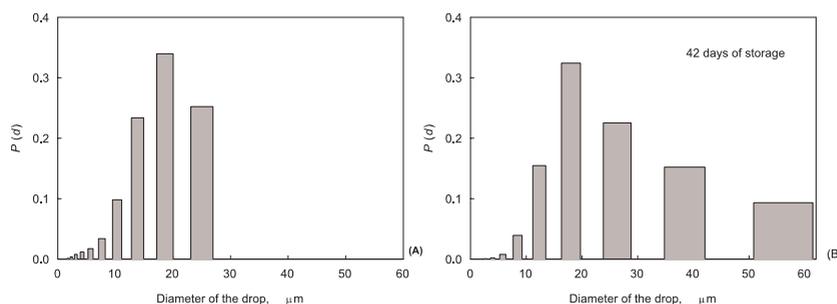

Figure 10. Histograms of the volume-weight drop-size distribution after: (A) 1 day of storage and (B) 42 days of storage for soybean oil-in-water emulsion, stabilized by 0.04 wt.% WPC (0.15 M NaCl, 0.01 wt.% NaN$_3$, natural pH).

missing in the freshly prepared emulsion (cf. Figure 10a and 10b). The presence of larger drops in the emulsion after 42 days of storage shows that some coalescence occurred during the storage period.

The experimental results, described above, show that the emulsion stability and the mean drop diameter increase with storage time for the emulsions prepared with 0.04 wt.% WPC. This result seems to be in an apparent contradiction with our previous results, which showed that the stability of WPC emulsions is lower for emulsions containing larger drops (at similar protein adsorption) [4,6]. On the other hand, we know that the stability of emulsions increases with the increase of protein adsorption. It was shown in our previous study that the protein adsorption was 1.9 mg/m^2 in fresh emulsions, stabilized by 0.04 wt.% WPC [6].

Taking into account the initial protein concentration, the measured mean surface-

volume diameter of the drops in the studied emulsions, and the protein adsorption, we could estimate the concentration of the remaining protein in the serum; for this particular emulsion we found 0.003 wt.%. This value is more than 10 times smaller than the initial protein concentration, which corresponds to a significant exhaustion of the protein solution, as a result of emulsion preparation. On the other hand, the coalescence of two drops in the emulsion leads to a net decrease of the oil-water interface, so the observed increase of the drop diameter upon storage (from 17 to 22 μm) would roughly correspond to an increase of the protein adsorption on drop surface from 1.8 to 2.2 mg/m^2 (assuming a constant protein concentration in the serum). Such an increase of the protein adsorption [6] would certainly lead to an enhancement of emulsion stability. To check the above hypothesis, we measured the protein concentration in the serum at the beginning of the storage period (1 day of storage) and after 35 days of storage and found no change. Taking into account the increased mean drop-size, we calculated that the protein adsorption, Γ, increased from 1.8 to 2.2$\pm$0.2 mg/m^2 during storage. Therefore, we explain the observed increase of emulsion stability at 0.04 wt.% WPC with an actual increase of protein adsorption on drop surface, as a result of the decreased specific surface area with time.

The histograms for 0.1 wt.% WPC showed no significant change of drop-size for storage time up to 50 days. Let us mention that the protein adsorption in emulsions stabilized by 0.1 wt.% WPC was measured to be around 2.2 mg/m^2, which was very close to the value obtained at the end of the storage period for emulsions prepared with 0.04 wt.% WPC [6]. The observed difference in the stability of emulsions prepared with 0.04 and 0.1 wt.% WPC could be explained by the different drop-size in these emulsions. The emulsions prepared with 0.1 wt.% WPC solution contain smaller drops and are, therefore, more stable at equal protein adsorption on the drop surface.

3.3 Comparison of EY and WPC stabilized emulsions

The experiments with both WPC and EY stabilized emulsions showed that the higher short-term stability corresponded to higher long-term stability, when the same type of emulsifier was used. To check how general is this relation, we compare the stability of EY and WPC emulsions. The dependence of P_{osm}^{cr} as a function of storage time for EY and WPC emulsions is shown in Figure 11. The obtained values of P_{osm}^{cr} after 1 day of storage for both concentrations of EY are more than 2 times higher than those for WPC emulsions. However, after 40 days of storage, a continuous oil layer was released on top of the EY emulsions, whereas the stability of 0.1 wt.% WPC emulsions was unchanged. Furthermore, the short-term stability of emulsions prepared with 0.04 wt.% WPC was lower than their long-term stability, while in the case of EY stabilized emulsions we observed a significant loss in stability with time. All these results show that higher short-term stability corresponds to higher long-term stability only for emulsions stabilized by the same type of emulsifier.
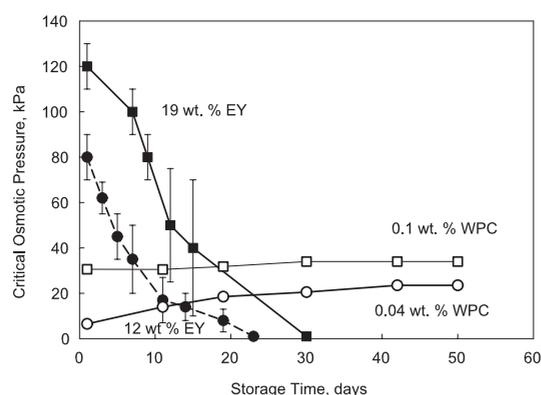
Figure 11. Comparison of the results for the critical osmotic pressure for emulsions stabilized by WPC and EY.

4   Main Results and Conclusions

The present paper presents an experimental study of the effect of storage time on the drop-size distribution and stability of EY and WPC emulsions. The main results could be summarized as follows:

No significant change in the drop-size distribution was detected for storage times up to 40 days for EY emulsions, whereas the emulsion stability decreased almost linearly with time. At the end of the storage period, a bulk oil layer appeared on the top of the emulsion even without centrifugation. Drop-drop coalescence occurred under shear in emulsions stabilized by 12 wt.% EY (stored $>$ 15 days) and 19 wt.% EY (stored $>$ 20 days). The emulsion destabilization is much more pronounced in the upper part of the EY emulsions, which is in contact with air (as compared to the lower part).

The stability of emulsions, prepared with 0.04 wt.% WPC, significantly increase with storage time. No changes in the emulsion stability and in the mean drop-size were observed for emulsions prepared with 0.1 wt.% WPC solution.

In conclusion, there is no general relation between the emulsion stability and the changes in the mean drop diameter upon shelf-storage of protein emulsions. The higher short-term stability corresponds to higher long-term stability for emulsions stabilized by the same type of emulsifier.

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References

[1] E. Dickinson, V. Galazka (1991) *Food Hydrocolloids* **5** 281.

[2] E. Bylaite, T. Nylander, R. Vensutonis, B. Jonson (2001) *Colloids Surfaces B: Bionterfaces* **20** 327.

[3] J.L. Klemaszewski, K.P. Das, J.E. Kinsella (1992) *J. Food Sci,* **57** 386.

[4] S. Tcholakova, N. Denkov, I. Ivanov, and B. Campbell (2002) *Langmuir* **18** 8960.

[5] S. Tcholakova, N. Denkov, D. Sidjakova, I. Ivanov, B. Cambell, "Interrelation between drop-size and protein adsorption at various emulsification conditions", *Langmuir*, submitted for publication.

[6] S. Tcholakova, N. Denkov, I. Ivanov, B. Campbell (2002) *Proceedings of the 3rd World Congress on Emulsion*, Lyon, France.

[7] J.S. Murday, R.M. Cotts (1968) *J. Chem. Phys.* **48** 4938.

[8] K.J. Packer, C. Rees (1972) *J. Colloid Interface Sci* **40** 206.

[9] Y. Mine, M. Bergougnoux (1998) *J. Agric. Food Chem*. **46** 2153.