Kinetics of Demulsification of Food Protein-Stabilized Oil-in-Water Emulsions

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Demulsification of food protein stabilized oil-in-water emulsions was determined as a function of time. Demulsification conformed to the empirical equation $q' = Q\ t/(B+t)$, where q' is the increase in moisture content of the down layer of emulsions at time t, Q is the maximum increase in moisture content, and B is the time required to gain Q/2. Emulsions separate water according to a second order rate law, which would indicate that flocculation is the rate determining step in demulsification.

Protein stabilized oil-in-water emulsions are found in various branches of the food industry. These include milk, cream, ice cream, salad dressings, mayonnaise, gravies and meat emulsions.

Two main approaches have been used to characterize the emulsifying properties of a protein—emulsifying capacity and emulsion stability. The former measures the maximum oil addition until phase separation occurs, whereas the latter measures the tendency for the emulsion to remain unchanged.

Proteins of different origins vary immensely in their ability to stabilize emulsions, reflecting their differences in composition, conformation and structural rigidity (1). Elizalde *et al.* (2) showed that emulsion instability could be predicted from the knowledge of the water and oil absorption capacity of a protein and the viscosity of the external phase. The relative importance of such factors in determining emulsion stability depended on the water-oil absorption index of proteins (3).

Different procedures have been used in estimating the stability of emulsions. Emulsion stability is commonly measured in terms of the amount of oil and/or cream separating from an emulsion during a certain period of time at a stated temperature and gravitational field (4-8). The time required for a specified degree of breakdown to occur is also used as a measure of stability (9,10). Several other methods have been used to measure emulsion stability. Turbidimetric measurements (11), light transmission by diluted ice cream emulsions (12), and conductivity measurements (13) are other techniques which have been used to measure emulsion stability.

Although the importance of studying the ability of proteins to stabilize emulsions has been reflected by the numerous investigations in this area, little effort has been expended in studying the overall process of demulsification. The present work was undertaken to study the kinetics of demulsification of food protein formulated emulsions and to identify quantitative parameters of emulsion stability.

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EXPERIMENTAL PROCEDURES

Materials. The following commercial soy protein isolates were used: Proteinmax 90 NB from Sambra S.A., Sao Paulo, Brazil; Purina Protein 760, 500 E and 710 from Ralston Purina Co., St. Louis, MO. Albumine bovine (AB) was from Sigma Chemical Co., St. Louis, MO. Sodium caseinate (SC) was from Lab. Argentinos Farmesa S.A., Argentina. Bean protein isolate (BPI) (*Phaseolus vulgaris* var. *Alubia*) was prepared according to Pilosof *et al.* (14). Meat soluble proteins (MSSP) were obtained according to Acton and Saffle (5), and freeze-dried. Gelatin (G) (food grade) was from Stauffer Rioplatense S.A., Argentina. Egg white powder (EW) was obtained by freeze-drying fresh egg white. Commercially available corn oil was from Refinerías de Maíz SAICF, Argentina.

Preparation of emulsions and measurement of demulsification. Emulsions were prepared by stirring 50 ml corn oil and 50 ml 1% (w/w) protein solution at 6,000 rpm for three minutes in a Griffing and George laboratory mixer. During emulsification the temperature was kept constant (4-5°C). When MSSP was tested, 3% (w/w) NaCl solution was used instead of distilled water. 10 ml emulsions were immediately distributed into test tubes and stored in a temperature controlled chamber at 45 ± 0.5°C. Demulsification was determined along the time interval 0-24 hours storage by the method of Acton and Saffle (5) by removing 5 ml emulsion from the bottom of the test tube and determining the moisture content (15). Demulsification at each storage time was expressed as the increase in moisture content, $q(t)-q_o$ of the bottom of test tubes, where $q(t)$ refers to the percent moisture after time t and q_o to the percent moisture of the freshly prepared emulsion. All tests were run in duplicate.

RESULTS

Figures 1 and 2 show the increase in moisture content of the bottom of emulsions as a function of time. All emulsions showed a restricted demulsification since most of the curves levelled off. The time for reaching this pseudo-equilibrium depended on the protein and varied from one hour (for PP500E) to approximately 10 hours (for sodium caseinate). However, rate of demulsification was initially rapid and slowed down as equilibrium was approached. The increase in moisture content at the point in which curves levelled off represented the maximum moisture increase (Q) for the down layer of emulsions.

Equation for fitting demulsification with time. In order to describe the curves in Figures 1 and 2, the following two-parameter equation was proposed,

$$q'(t) = q(t) - q_o = Qt/(B + t) \qquad [1]$$

where $q(t)$ refers to the moisture content at time t; q_o refers to the moisture content of the freshly prepared

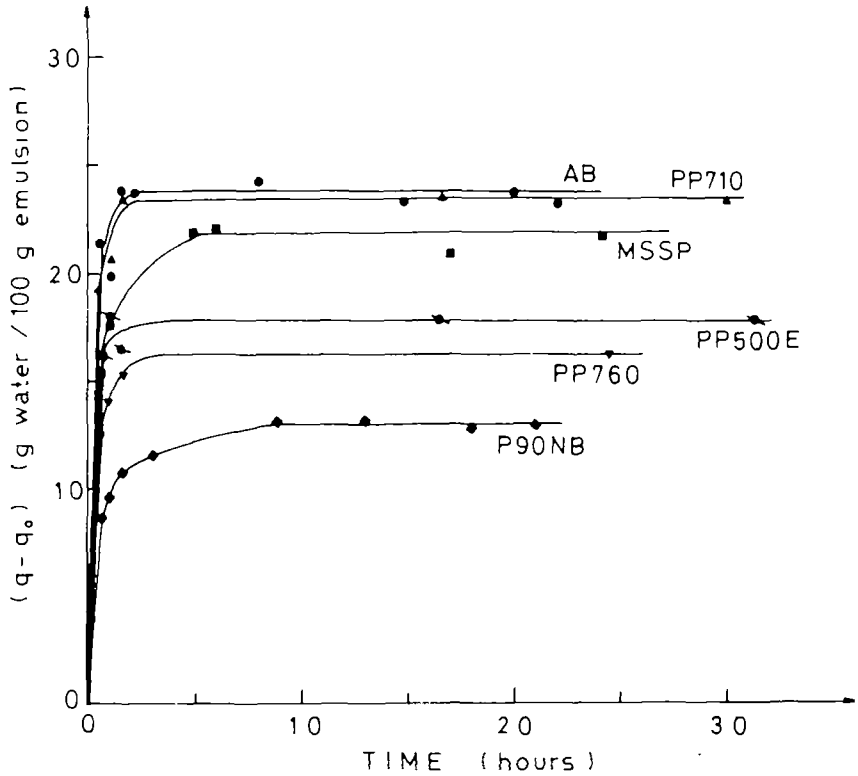

FIG. 1. Demulsification of various protein-stabilized emulsions as a function of time of storage at 45°C. AB: albumin bovine; PP710: Purina protein 710; MSSP: meat salt-soluble proteins; PP500E: Purina protein 500E; PP760: Purina protein 760; P90NB: Proteinmax 90NB.

emulsion; Q refers to the maximum moisture increase; and B refers to the time needed to gain half the maximum moisture increase (Q/2).

In order to find the best statistical parameters Q and B which give the best fit of experimental data (q'_i, t_i), the following function was demanded to be a minimum:

$$\chi^2 = \sum_{i=1}^{n} (q'_i - \hat{Q}\, t_i/(\hat{B} + t_i))^2 \qquad [2]$$

For that purpose a program for nonlinear least squares analysis (16) was used; data were processed on a IBM PC.

In order to obtain the estimator of the standard deviations of $\hat{Q}$ and $\hat{B}$, the following covariance matrix was evaluated:

$$c \approx \begin{pmatrix} \hat{\sigma}Q^2 & \hat{\sigma}QB \\ \hat{\sigma}QB & \hat{\sigma}B^2 \end{pmatrix} \qquad [3]$$

where $\hat{\sigma}Q$ and $\hat{\sigma}B$ represent the estimators of the standard deviations of $\hat{Q}$ and $\hat{B}$, and $\hat{\sigma}QB$ represents the correlation between $\hat{Q}$ and $\hat{B}$.

In order to evaluate the goodness of fit of Eq. [1] as applied to the experimental data, the "relative absolute percent error" was computed:

$$\epsilon\% = 100/n \sum_{i=1}^{n} |\, q'_i - \hat{Q}\, t_i/(\hat{B} + t_i)|/q'_i \qquad [4]$$

Table 1 shows the best statistical parameters $\hat{Q}$ and $\hat{B}$, the estimators of their standard deviations and the goodness of fit of Eq. [1] as applied to the different protein stabilized emulsions. Eq. [1] was able to fit the data well, as shown from the "relative absolute percent error" values, which ranged between 1 and 7%. $\hat{B}$ was the most uncertain parameter on comparison with $\hat{\sigma}B$; this result is probably due to the fact that $\hat{B}$ is a very short time. Figure 3 shows the comparison between the experimental curves vs the mathematically regenerated curves based on Eq. [1] for several emulsions. From this it can be seen that the agreement is fairly good.

Kinetics of demulsification. Rate of demulsification could be derived by differentiating Eq. [1] with respect to time which yields:

$$dq'/dt = (1/BQ)(Q - q')^2 \qquad [5]$$

where (Q-q') represents the amount of water that must still be gained by the down layer of emulsions to reach maximum demulsification. $(BQ)^{-1}$ represents the specific rate constant K for the demulsification process. Therefore, K could be calculated as:

$$\hat{K} = (\hat{Q}\,\hat{B})^{-1} \qquad [6]$$

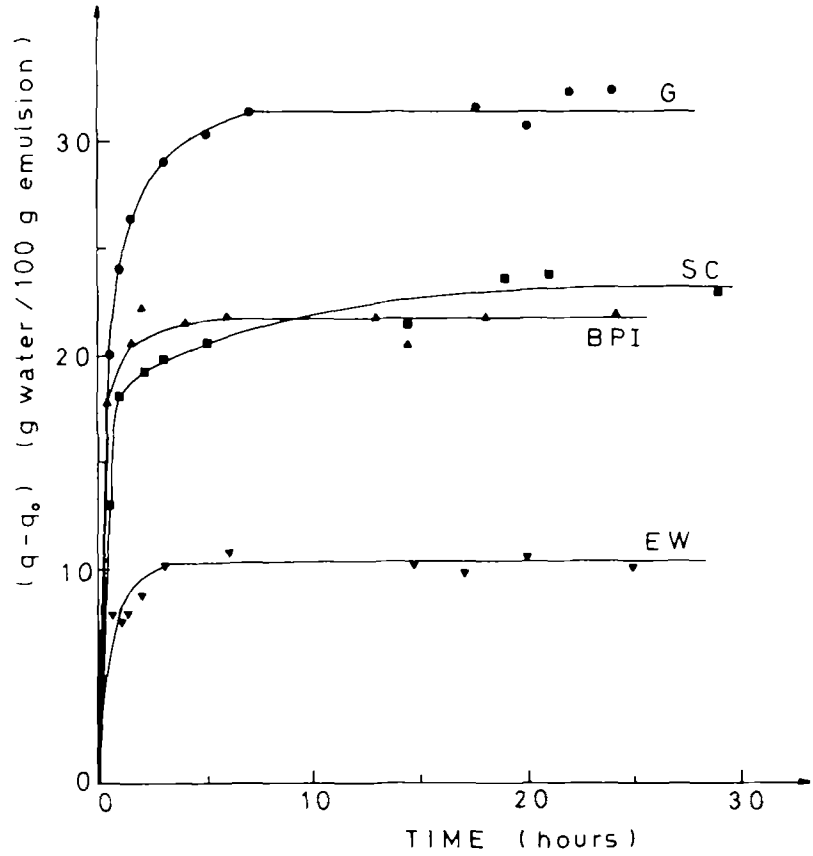

FIG. 2. Demulsification of various protein-stabilized emulsions as a function of time of storage at 45°C. G: gelatin; SC: sodium caseinate; BPI: bean protein isolate; EW: egg white.

TABLE 1

Parameters Which Describe the Demulsification of Different Protein-Stabilized Emulsions as a Function of Time

Protein	No data	$\hat{Q} \pm \hat{\sigma}Q$ g water/100 g emulsion	$\hat{B} \pm \hat{\sigma}B$ (hr)	ϵ%
EW	10	10.3 ± 0.2	0.246 ± 0.006	7
P90NB	8	13.21 ± 0.05	0.342 ± 0.002	3
PP760	5	16.5 ± 0.2	0.153 ± 0.001	2
PP500E	5	17.70 ± 0.01	0.045 ± 0.003	4
MSSP	6	21.80 ± 0.02	0.213 ± 0.003	6
BPI	9	21.7 ± 0.2	0.099 ± 0.001	3
AB	8	23.7 ± 0.4	0.073 ± 0.001	4
PP710	5	23.7 ± 0.7	0.113 ± 0.001	2
SC	9	23.2 ± 0.2	0.370 ± 0.003	4
G	10	32.1 ± 0.1	0.312 ± 0.001	1

The estimator of the standard deviation of $\hat{K}$ was calculated as:

$$\hat{\sigma}K^2 = (\partial\hat{K}/\partial\hat{Q})^2\,\hat{\sigma}Q^2 + (\partial\hat{K}/\partial\hat{B})^2\,\hat{\sigma}B^2 + 2(\partial K/\partial\hat{Q})(\partial\hat{K}/\partial\hat{B})\,\hat{\sigma}QB \quad [7]$$

The specific rate constants of demulsification of emulsions and the estimators of the standard deviations of $\hat{K}$ are shown in Table 2. An acceptable accuracy in the K-values was obtained.

Initial rate of demulsification. By differentiating Eq. [1] with respect to time and evaluating it at t = 0, the initial rate of demulsification could be derived and yields:

$$R_o = (dq'/dt)_{t\to o} = \hat{Q}/\hat{B} \quad [8]$$

R_o values of the different emulsions are included in Table 2.

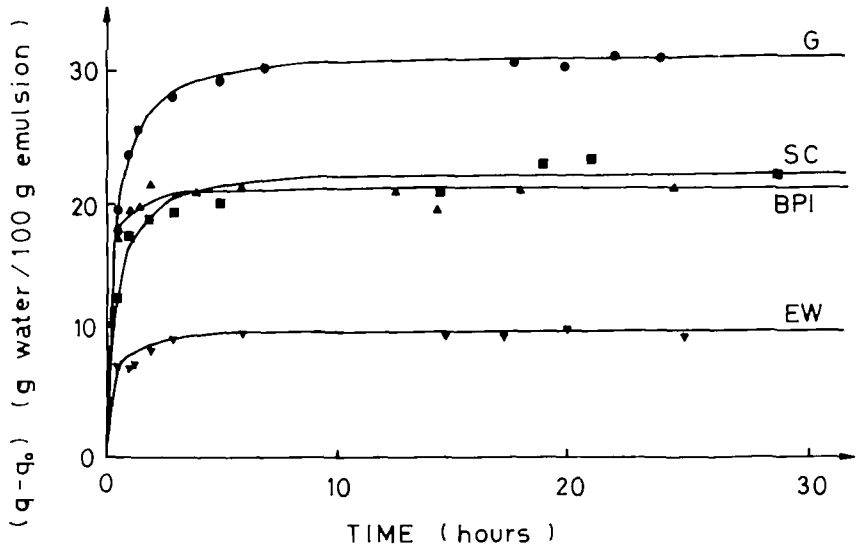

FIG. 3. Comparison of predicted (solid lines) and experimental (single points) demulsification curves of various protein stabilized emulsions. G: gelatin; SC: sodium caseinate; BPI: bean protein isolate; EW: egg white.

TABLE 2

Specific Rate Constants and Initial Rate of Demulsification for Different Protein-Stabilized Emulsions

Protein	$\hat{K} \pm \hat{\sigma}K$ $\left(\frac{\text{g water}}{100\text{ g emulsion}\,h}\right)^{-1}$	R_0 $\frac{\text{g water}}{100\text{ g emulsion}\,h^{-1}}$
EW	0.39 ± 0.02	42
P90NB	0.22 ± 0.02	39
PP760	0.40 ± 0.01	108
PP500E	1.25 ± 0.03	393
MSSP	0.215 ± 0.001	102
BPI	0.46 ± 0.03	220
AB	0.57 ± 0.01	325
PP710	0.38 ± 0.02	210
SC	0.117 ± 0.001	63
G	0.0996 ± 0.0001	103

DISCUSSION

Four quantitative criteria of emulsion stability may be used in order to compare the capacity of the various proteins tested: First, the maximum amount of water gained by the down phase of emulsions (Q); second, the time needed to gain half the maximum amount of water (B); third, the specific rate constant of demulsification (K), and fourth, the initial rate of demulsification (R_0).

Q is a good criterion of emulsion stability if we are assessing the maximum amount of demulsification that would occur during long term storage of the emulsion. From this point of view, egg white showed the best stabilizing properties while the gelatin-based emulsion showed the highest degree of demulsification.

However, the Q-value alone does not entirely describe the instability of emulsions since it does not indicate the rate of demulsification. Either K or B values can be used in order to characterize the rate of demulsification. As shown in Eq. [6], K is determined by either Q and B-values; however, changes in Q were always smaller than those for B-values so that the rate constants were primarily determined by B values as indicated the correlation obtained ($R = 0.763$; $P < 0.01$) between specific rate constants and B values of the various emulsions. PP500E, which had the lowest B value, showed the highest rate constant of demulsification. Gelatin, despite having the highest Q value, showed the lowest rate constant due to its low B value. MSSP and sodium caseinate also showed very low rates of demulsification.

If the interest is focused on the first stages of demulsification, R_0 should be the most adequate index of emulsion instability as it indicates the initial rate of demulsification. As shown in Table 2 for gelatin and egg white, initial rates of demulsification are not always correlated with the rate constant of the overall process of demulsification.

Kinetic processes which might determine the overall rate of demulsification include creaming, flocculation and coalescence. Certainly, in practical systems all three processes may appear to occur either simultaneously or sequentially in any order (17). The relative rate constants of the three processes would determine which step is rate determining in the overall demulsification process.

Rates of sedimentation (or creaming) depend on density differences, molecular and micellar weights, temperature, and presence or absence of swamping electrolytes (17). The rate of flocculation is determined by the balance between electrical repulsion and van der Vaals attraction, which gives the potential energy between the drops as a function of the distance separation according to the DLVO theory, and the forces of thermal diffusion and convection tending to bring them into contact. The rate of coalescence depends on the rupture of the adsorbed film of protein at the oil-water interface, which will depend primarily on their thickness and on its viscoelastic properties.

A zero order rate law (18) indicates that coalescence at the interface between bulk oil and emulsion is the rate-determining step of the overall process of demulsification. A first order rate law indicates that the intrinsic rate of coalescense between oil drops within the body of the emulsions is the slowest step, and a second order rate law that the rate of flocculation is the determinant of the overall rate of demulsification. In the case of the protein-stabilized emulsions studied here, the flocculation step would be the determinant of the overall rate of demulsification, as indicated by its obedience to a second order rate law.

ACKNOWLEDGMENTS

The authors wish to thank the Subsecretaría de Cienca y Tecnología, the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina, and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires for their financial support.

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