Influence of pH on surface properties of aqueous egg albumen solutions in relation to foaming behaviour

Marianne Hammershøj,[1]* Albert Prins[2] and Karsten B Qvist[3]

[1]Danish Institute of Agricultural Science, Dept of Animal Product Quality, POB 50, 8830 Tjele, Denmark
[2]Wageningen Agricultural University, Dept of Food Technology and Nutritional Sciences, Food Physics Group, POB 8129, 6700 EV Wageningen, The Netherlands
[3]The Royal Veterinary and Agricultural University, Dept of Dairy and Food Science, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

Abstract: The surface properties of aqueous egg albumen protein solutions (0.1 g litre^{-1}) were studied at pH values of 4.8, 7.0, 9.2 and 10.7 and related to foaming behaviour such as bubble size distribution, overrun and drainage. By measurements far from equilibrium of dynamic steady state surface dilation using the overflowing cylinder technique, egg albumen showed ability to slow down surface expansion and to lower the dynamic surface tension. The pH-effect was small, but at pH 4.8 the film length, at which a motionless surface was created, was longer than at higher pH indicating a somewhat more rigid surface at low pH. Near equilibrium sinusoidal surface area deformation resulted in relatively high moduli of egg albumen, with a significant effect of pH. The surface modulus E showed at pH 4.8 an increase in the course of time, but at higher pH it was constant. Large deformation of egg albumen surface was not destructive, and for all pH values the surface behaved viscoelastic, with highest loss modulus E'' and tan θ values at pH 4.8. Surface deformation frequency sweeps revealed the relaxation processes to be relatively slow at pH 4.8 and faster at pH 7.0–10.7. Foamability measured as overrun of foam as a result of shaking and stirring was highest at pH 4.8 and lowest at pH 10.7. Foam stability against drainage was best at pH 7.0 after 30 min, but at a long-term scale foam at pH 4.8 was most resistant to drainage. Foam samples were subjected to microscopy and image analysis. The smallest bubbles were found at pH 4.8 (mean diameter 142 μm) and the largest at pH 7.0 (mean diameter 328 μm). In conclusion, the foaming behaviour of an aqueous egg albumen solution at pH 4.8 can be related to dynamic surface properties as follows: the more rigid behaviour of the surface at this pH favours a small bubble size and slow drainage of liquid from the foam. The high overrun at this pH can be explained by a lower dynamic surface tension, but also here film stability during foam making can be promoted by a more rigid liquid surface.

Keywords: surface properties; protein; egg albumen; foam; pH; surface tension; surface rheology

INTRODUCTION

In food products, where a gas is dispersed into a liquid, the foaming ability of the system is an important functional property. Egg albumen is widely used in different food products as a foaming agent and is known to have excellent foaming properties. The dry matter of egg albumen contains approximately 80% protein, but the knowledge of the role of these proteins, in relation to foamability and foam stability and how the surface properties of egg albumen films are acting under different conditions, is limited.

Foam creation is a dynamic process during which the surface of the protein film surrounding the gas bubbles is being compressed and expanded. The foam can be created by different methods: whipping/stirring, bubbling/sparging and shaking, varying as regards amount of gas available for incorporation. The stability of the foam throughout time and handling is influenced by the ability of the air bubble surface to resist expansion and compression, and to slow down liquid drainage from the foam. Foam stability can be measured in numerous ways and most methods are empirical, even though more fundamental methods as conductivity,[1,2] current input[3] and video image analysis[4] of foam have been applied.

Destabilising mechanisms in protein foams can be divided into three; (1) liquid drainage, where liquid flows from the foam as a consequence of gravity force and the foam dries out, (2) bubble coalescence, where rupture of the film between two bubbles causes them to merge into one large bubble, and (3) bubble disproportionation, where gas diffusion from small bubbles, due to a higher gas pressure into large bubbles, results in shrinkage of small bubbles and

* Correspondence to: Marianne Hammershøj, Danish Institute of Agricultural Science, Dept of Animal Product Quality, POB 50, 8830 Tjele, Denmark
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growth of large bubbles in course of time.[5] These mechanisms act by forces working both parallel and perpendicular to the surface.

Drainage causes a flow parallel to the surface of the film causing a heterogeneous deformation. Liquid flow around the bubble surface will in the upper part result in expansion and in the lower part in compression. The drainage is counteracted by the ability of the liquid to create a surface tension gradient and thereby reduce the viscous flow by a counterflow of surface and underlying fluid from areas of low to areas of high surface tension, also called the Marangoni effect.[6]

The stability of a foam is affected by electrostatic interactions between the molecules in the film.[7] Properties that stabilise foam by intermolecular interactions in the surface film are both repulsion of adjacent films, attraction of counter-ions acting as polymeric components in the film, and formation of thicker and stronger films around the isoelectric point, pI.[7] In the pH region near pI of a protein the net charge is zero, and electrostatic forces between molecules are minimal. This can favour non-electrostatic intermolecular bonding and thereby increase the stability of the foam.[8]

In a foam the surfaces of the bubbles are separated from each other by thin films and plateau borders, where films meet at an angle of 120°. The proteins in the surface may interact in different ways to create a network, by van der Waals forces, electrostatic, hydrophobic, hydrogen bonds, and even covalent disulphide bonds.[7] Electrostatic repulsion or steric hindrance between proteins can be imagined to create a thick film, as the molecules cannot approach each other and only pack to a minor extent.[7] This is contributing to retarded gas diffusion, disproportionation, coalescence and film rupture.

Formation of a protein network can proceed by thiol and disulphide exchange, which is very rapid at neutral to alkaline pH,[9] and by oxidation of cystein residues into disulphide bonds. These mechanisms can in food systems be expected to play a role in the inter- and intramolecular actions of the proteins. Both reduction of intersubunit disulphide bonds of soy protein and formation of disulphide bonds in the air/water interface of β-lactoglobulin are reported to improve foam stability,[7] which means that the role of disulphide bonds in protein foams is ambiguous.

At high pH values the fast exchange of the thiol- and disulphide bonds can influence the surface properties of the protein film, as split-up of disulphide bonds increases the flexibility, opportunity of orientation, and unfolding of the molecule in the interface. Especially for the ovalbumin, which is the only protein in egg albumen known to have free SH-groups, such exchanges may play a role by stabilising a protein film by polymerisation and thereby influence the surface properties.

To study the foam making and also the foam stability against rapid drainage the measurement of surface tension of an expanding liquid surface obtained under dynamic conditions will give information of the ability to lower surface tension and to build a surface tension gradient counteracting the liquid drainage.[10]

As the egg albumen consists of several proteins with a wide range of pI from 4.1 (ovomucoid) to 10.7 (lysozyme), it is worth knowing about the surface behaviour of this complex protein mixture and the variation with pH. A range of net charges will exist at different pH values in the egg albumen. The egg albumen proteins known to contribute either to foam building or foam stability are listed in Table 1. Egg albumen contains 40 known proteins, with about half as minor components.[11]

The ability of the egg albumen proteins to decrease surface tension, as seen in Table 1, can contribute to stabilising a foam, as surface-active agents which decrease the surface tension has a stabilising effect on thin films.[12] To prevent breakdown by coalescence a strong Marangoni effect, high disjoining pressure and film elasticity can also be important.

The disproportionation of air bubbles (Ostwald ripening) is the consequence of gas transportation from small air bubbles into larger ones and is driven by the Laplace pressure difference $\Delta P = 2\gamma R^{-1}$, where P is the pressure, γ the surface tension, and R the bubble

Table 1. Major proteins in egg albumen, composition, size and properties[11,32–34]

Protein	% of albumen protein	Composition	Mw (kDa)	pI	Surface tension[a] (mNm^{-1})	Function in foods
Ovalbumin	54.0	386 residues, 4-SH, 1 -SS-	45	4.5–4.7	51.8	Denaturation in foam, heat labile
Conalbumin	13.0	705 residues, 9-SS- in C-domain 6-SS- in N-domain	76.6-80	6.1–6.6	42.4	Heat labile
Ovomucoid	11.0	186 residues, 7-9 -SS- groups	27.3	4.1	39.0	Heat- and acid stability
Ovomucin	1.5–3.0	Not sequenced, -SS- groups, α1, α2 and β-ovomucin	α_1 135 α_2 210 β 720	4.5–5.0	—	Stabilises foam, increases viscosity
Lysozyme	3.4–3.5	129 residues, 4 -SS- groups	14.3	10.7	42.0	Interacts with ovalbumin
Ovoglobulins G2 and G3	4.0–4.2	Not sequenced, 2 genetic variants	G2:47 G3:50	5.5	45.4	denatures fast

[a] Determined on a Fisher Surface Tensiometer model 20.[31]

radius.[13] As the equilibrium solubility of gas in a liquid is proportional to P, the smaller bubbles will have a higher gas pressure and therefore higher gas concentration, which causes diffusion of gas from small into large bubbles. The disproportionation process can be stopped, when the surface tension of the smaller bubble has a value low enough to compensate for the decrease in radius of the bubble, so the pressure difference will remain constant.[13] This has been calculated to be the case when the surface has complete elastic behaviour and the surface modulus $E > \frac{1}{2}\gamma$. The dilational modulus $E = A\ \mathrm{d}\gamma(\mathrm{d}A)^{-1} = \mathrm{d}\gamma\ (\mathrm{dln}A)^{-1}$ describes the relation between a dilational deformation change in surface area A by dA and the resulting dγ,[14] in popular terms called the surface stiffness or resistance to deformation. The modulus E is composed by an elastic component—the storage modulus E'—and a viscous component—the loss modulus E''; modulus $E = \sqrt{E'^2 + E''^2}$ which all are components that can be measured by the Ring Trough Technique.[13]

The ratio between the loss modulus and the storage modulus equals the tangent of the loss angle θ (phase difference) between a sinusoidal dA and the resultant sinusoidal dγ, $\tan\theta = E''E'^{-1}$. The viscous modulus E'' is a product of the angular frequency ω (rad s^{-1}) and the surface dilational viscosity η_d (mN s m^{-1}); $E'' = \omega\eta_d$. This surface dilational viscosity is not equal to the surface dilational viscosity under steady state expansion or compression $\eta_d{}^s = (\gamma_{dyn} - \gamma_e)\ (\mathrm{dln}A\ (\mathrm{d}t)^{-1})^{-1}$.

Foamability, measured as foam volume, has shown to correlate well with the ability to decrease the dynamic surface tension,[10] and high dynamic surface tension is unfavourable for foam stability during foam making.[15]

A number of studies of surface behaviour and properties of single protein systems has been performed,[10,16,17] however, egg albumen, which is often used in food products, contains a wide range of different proteins present, and it seems obvious to understand the properties of this complex protein source.

The aim of this study is to examine the surface properties of egg albumen protein solutions under dynamic and near equilibrium circumstances at pH 4.8 (near pI of ovalbumin and ovomucin), 7.0 (fresh egg), 9.2 (aged egg) and 10.7 (pI of lysozyme) in relation to foamability, foam stability and bubble size distribution of foam. In this wide pH-range the electrostatic interactions between proteins, the reactivity of thiol-groups, possible changes in conformation and aggregation can be expected to influence the foaming properties.

MATERIALS AND METHODS

Materials

Dried egg albumen powder obtained from a commercial company (NIVE (OP2G from NIVE, Nunspeet Holland Eiprodukten BV, Postbus 113, 8070 AC Nunspeet, The Netherlands)) was dissolved in an aqueous buffer to a protein concentration of 0.1 g litre^{-1} . Four different buffer solutions were used with the following pH values: 0.2 M CH_3COONa pH 4.8, 0.2 M $KH_2PO_4/Na_2HPO_4 2\ H_2O$ pH 7.0, 0.2 M TRIS (tris[hydroxymethyl]aminomethane)/NaCl pH 9.2 and 0.2 M Glycine/NaOH/NaCl pH 10.7. All buffers were supplied with 0.1 M NaCl in order to reach a ionic strength for 'salting in' the egg albumen proteins. Especially the solubility of ovomucin requires a ionic strength above 0.1.[18] This was controlled by conductivity measurements of the buffers, as low ionic strength would cause aggregation of the proteins. All the egg albumen powder solutions prepared were clear and showed no aggregation. In order to obtain a proper wetting of the egg albumen powder, it was dissolved 3.5–4 h prior to measurements. All solutions and measurements were performed at 21 ± 2 °C.

Methods

The overflowing cylinder

To measure the dynamic surface properties far from equilibrium but under steady state dilation, an overflowing cylinder apparatus was used.[10] The apparatus is composed of two cylinders: an inner cylinder, where the liquid flows to the top and over the rim and falls down as a film on the outside wall into the outer cylinder, where the liquid in a closed loop is pumped into the bottom of the inner cylinder again. The cylinder used had a diameter of 0.03 m, and the flow rate was constant at 3.3 10^{-5} m^3 s^{-1}.

The dynamic surface tension γ_{dyn} of a constantly expanding surface was measured near the centre by a glass Wilhelmy plate connected to a transducer and a recorder. The surface expansion rate $\mathrm{dln}A\ (\mathrm{d}t)^{-1}$ was calculated as $2v_r r^{-1}$ from surface velocity, v_r, measurement of inert talcum powder particles on the expanding surface at a radius r from the centre. The equilibrium surface tension γ_e was measured on the surface in the inner cylinder in the absence of a liquid flow, and from these measurements the surface dilational viscosity $\eta_d{}^s = (\gamma_{dyn} - \gamma_e)\ (\mathrm{dln}A\ (\mathrm{d}t)^{-1})^{-1}$ was calculated. All measurements were performed in triplicates.

During these measurements the effect of the length, L, of the falling film on the expanding surface was examined. It is known for surfactant solutions that at high L values the γ_{dyn} and $\mathrm{dln}A\ (\mathrm{d}t)^{-1}$ do not depend on L. The γ_{dyn} is always higher than γ_e, and $\mathrm{dln}A\ (\mathrm{d}t)^{-1}$ is higher than the value obtained for pure water, which for this apparatus is about 5 s^{-1}. At lower L values, however, the surfactant in solution is able to slow down the expansion rate of the surface to below the value of water. The length of the falling film, where the value of $\mathrm{dln}A\ (\mathrm{d}t)^{-1}$ crosses the value obtained for pure water (5 s^{-1}), and where γ_{dyn} becomes lower than that of pure water (72 mN m^{-1}), is called the critical length, L_{crit}. This value is very dependent on the surface active agent. Some proteins are able to build up a stagnant surface layer, which can slow down the

$d\ln A\ (dt)^{-1}$ by a factor 10^3.[17] The higher the value of L_{crit} the more the surfactant is able to keep the surface motionless.

The ring trough

The surface tension and the dilational modulus under conditions near equilibrium, but not under steady state dilation of the surface was examined by dynamic measurements in the Ring Trough apparatus.[19,20] In a trough a surface enclosed by a ring is being expanded and compressed by moving the ring up and down by sinusoidal oscillation, and the surface tension variation due to variation in surface area is measured near the centre by a Wilhelmy plate.

Time effect on the surface properties was measured by recordings each 2.5 min, until the dilational modulus had stabilised, or for maximum 60 min. The experimental details for these recordings were an amplitude of 1 mm of the ring resulting in $dA\ A^{-1} = 0.037$, frequency 100 mHz which equals $\omega = 0.628$ rad s^{-1}, and continuous ring movement.

By applying an increasing surface area deformation from $dA\ A^{-1}$ of 0.0001 to approximately 0.09 and thereafter decreasing it again, the linear region, where the surface dilational modulus E is independent of deformation (within 90% of the value at low strain) together with a possible history effect of large deformation on the surface, can be evaluated.

A frequency sweep was performed to study the effect of time-scale on surface properties. Due to the relaxation processes taking place, viscous response will dominate at low frequency (long time scale), and elastic response at high frequency. From the surface dilational viscosity η_d ($mN\,s\,m^{-1}$) it can be detected, whether the surface displays 'shear thinning' or 'shear thickening' behaviour, although the present type of surface deformation is not shearing. Measurements with the Ring Trough were performed in two replicates.

Foamability and stability

To evaluate the foamability and the stability of the obtained foams at different pH values, foaming experiments were performed in glass tubes by shaking for 25 s, all done in three replicates. The foaming behaviour was evaluated by foamability measured as relative overrun $= v_0\ v_i^{-1}$, foam stability $= v_{30}\ v_0^{-1}$, and liquid drainage $= 1 - ((v_i - vl_{30})\ \ (v_i - vl_0)^{-1})$, where v_i = initial liquid volume, v_0 = foam volume at 0 min, v_{30} = foam volume at 30 min, vl_0 = volume of liquid at 0 min, and vl_{30} = volume of liquid at 30 min.

Spreading particles

Examination of the egg albumen solutions by the Free Falling Film Technique[13] gives information about the presence of hydrophobic and spreading particles, which can cause spreading at the surface and hole formation in the film and thereby cause bubble coalescence in a foam. The falling film is observed under stroboscopic light, by which hole formation and light twinkling due to particles are detectable.

Bubble size distributions of foam

Additional to foam produced by shaking, the bubble size distribution, foamability and stability of foam produced by stirring, were also measured. A liquid sample of 100 ml was stirred for 60 s at 2500 rpm with at fan-shaped whisk in a cylindrical glass with a tip speed of 6.0 m s^{-1}. Immediately after foam formation a foam sample was applied to an object-glass with a concave slit and examined. The bubbles were visualised in a microscope (Zeiss Axioskop) connected to the PC programme ColorVisionI (ColorvisionI version 1.17, Cocoon software, Buitenweg 28, 4707 SX Roosendaal, The Netherlands). Within 0–6 min after foam formation pictures were taken by video camera, and on these pictures the diameter d of bubbles was measured by PC-Image (PC-Image version 2.2, Foster Findlay Assoc. Ltd, Newcastle Technopole Kings Manor, Newcastle upon Tyne, NE1 6PA, UK). Bubble surface area and bubble volume were calculated as $A = \pi\ d^2$ and $V = 6^{-1}\ \pi\ d^3$, respectively. For each sample two replicates of 20 pictures of bubbles were analysed with 130–600 bubbles per replicate. The distribution of bubble sizes was made by diameter classes ranging from 0–25 μm, 25–50 μm, 50–100 μm, and so on by intervals of 50 μm until the maximum bubble diameter measured.

The mean bubble diameter distributed on the basis of number of bubbles is given as d_{10}, and the mean diameter by volume/surface ratio is given as d_{32} from the equation: $d_{ab} = (S_a/S_b)^{(1/a-b)}$, where for d_{10}, $a = 1$ and $b = 0$ and for d_{32}, $a = 3$ and $b = 2$. The value $S_n = \Sigma_{i=1}\ N_i\ d_i^n$, and N_i is the number of bubbles in class i, and d_i is the diameter of bubbles in class i. The relative standard deviation of the mean diameter measures the relative width of the distribution and is in general given by $C_n = \sqrt{(S_n S_{n+2}/S^2)} - 1$, and for d_{32} the relative standard deviation is $C_2 = \sqrt{(S_2 S_4/S_3^2)} - 1$, where low values indicate a homogenous foam and high values a wide distribution and large difference between the d_{10} and d_{32}.

RESULTS

Dynamic measurements by the overflowing cylinder

The measured values of the critical length of the falling film L_{crit}, as a function of pH showed only small differences as seen by data presented in Table 2. At the low pH 4.8 the value of L_{crit} was in general highest, which means that the weight of the free falling film is creating a surface tension gradient of the expanding top surface at a longer film length. Even though the differences are small and not significant, they tend to indicate that the egg albumen builds a rigid surface film more readily at this pH.

The γ_e of egg albumen solution in Table 2 showed only minor pH effects resulting in values in the range of 39.5–42.5 $mN\,m^{-1}$. Other surfactants such as

Table 2. Critical film length, L_{crit}, mean±SD, and surface tension at equilibrium γ_e of egg albumen solution (0.1 g litre^{-1}), n=3

pH	*4.8*	*7.0*	*9.2*	*10.7*
L_{crit} (cm)	2.1±0.05	1.9±0.2	2.0±0.05	1.9±0.1
γ_e (mN m^{-1})	41–41.5	39.5	39.5–42.5	41

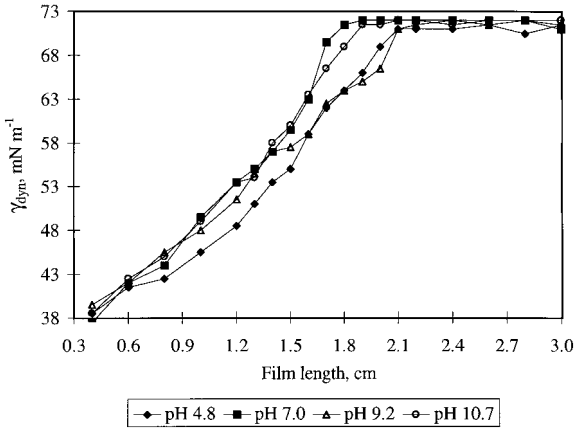

Figure 1. Dynamic surface tension γ_{dyn} as function of wetting film length L for egg albumen protein solutions (0.1 g litre^{-1}) at different pH values.

Teepol and sodium-caseinate have γ_e values of 28 mN m^{-1} and 46 mN m^{-1}, respectively.[10]

In Fig 1 the effect of pH on γ_{dyn} as a function of film length is shown. The differences are again quite small, but a tendency to obtain lower surface tension values at $L < L_{crit}$ for pH 4.8 compared to pH values for 7.0–10.7 was consistent in all experiments, both when the film length was decreased and increased.

The effect of film length and pH of the egg albumen solutions on the surface expansion rate is shown in Fig 2, with $d\ln A\ (dt)^{-1}$ on a log scale. Also here pH 4.8 reached lower values of $d\ln A\ (dt)^{-1}$, which at a film length of 1.6 cm is a magnitude lower than at other pH values. This indicates that at low pH the surface's ability to slow down the expansion and build up a film is higher, which correlates with the lower γ_{dyn} in Fig 1.

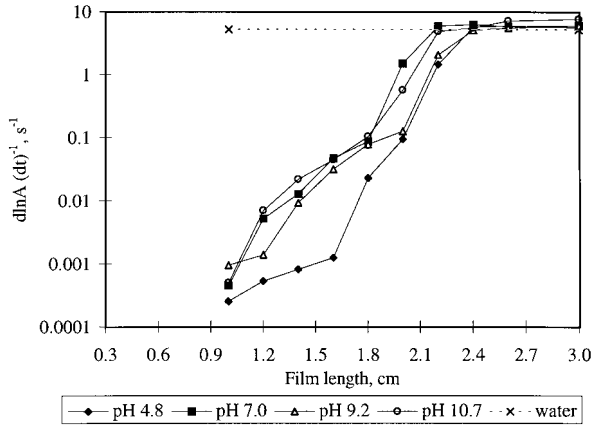

Figure 2. Surface expansion rate $d\ln A\ (dt)^{-1}$ on log scale as function of wetting film length L for egg albumen solutions (0.1 g litre^{-1}) at different pH values and for water.

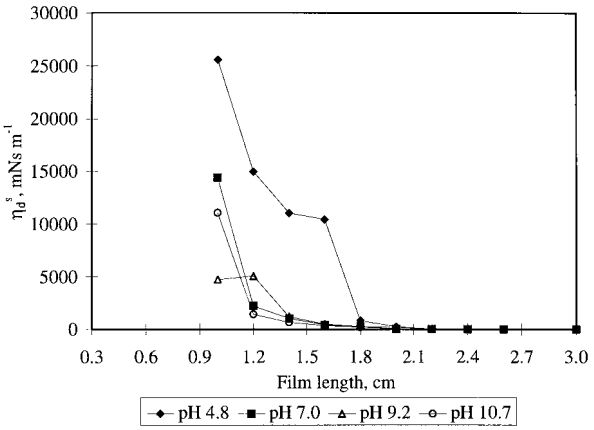

Figure 3. Surface dilational viscosity η_d^s as function of wetting film length L for egg albumen solutions (0.1 g litre^{-1}) at different pH values.

In Fig 3 is given the surface dilational viscosity η_d^s in relation to the film length under steady state dilation. The values of η_d^s at high film lengths were in the range 4–6 mNs m^{-1}. The curves of especially pH 4.8 and pH 9.2 show large differences in viscosity at low film length. The results show the egg albumen creating a stiff surface at pH 4.8, and although such a surface is build up also at pH 9.2, it appears a magnitude 'less stiff'.

Measurements of the surface dilational modulus

The measurements were performed using a Ring Trough by measuring the effect of time, amplitude, and frequency on the surface modulus E and surface tension γ at each pH.

In Fig 4 the effect of time and pH on the surface dilational modulus E is shown for time scales up to 60 min. There appeared to be no time effect at pH 7.0 and pH 9.2, where the dilational modulus E was constant at 29 mN m^{-1} and 22 mN m^{-1}, respectively. But at pH 4.8 the modulus E increased with increasing time from 32–51 mN m^{-1}. At pH 10.7 the surface modulus E increased slightly from 34–38 mN m^{-1} with increasing time.

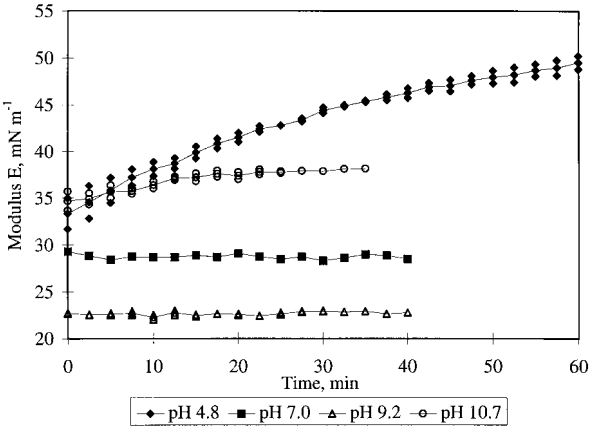

Figure 4. Effect of time and pH on surface dilational modulus E of egg albumen solutions (0.1 g litre^{-1}), surface deformation $dA\ A^{-1}$=0.037, frequency ω=0.628 rad s^{-1}.

	pH			
	4.8	*7.0*	*9.2*	*10.7*
Increasing time				
E' (mN m^{-1})	3.9→6.1	2.0	1.7	2.8
tan θ	0.15→0.11	0.07	0.08	0.07
γ (mN m^{-1})	46	48→43	44	50→47
Increasing deformation				
max dA A^{-1} of linear region[a]	0.036	0.09	0.09	0.09
Increasing deformation frequency of 0.038→2.413 rad s^{-1}				
E' (mN m^{-1})	39→59	25→34	13→26	32→39
E'' (mN m^{-1})	7.3→4.8	3.7→1.2	2.5→0.8	5.4→1.1
tan θ	0.17→0.08	0.15→0.04	0.18→0.04	0.17→0.03
η_d, (mNs m^{-1})[b]	90→5	52→1	40→1	65→1

Table 3. Dynamic measurements of near equilibrium deformation in the Ring Trough of egg albumen surface at four different pH values, with effect of time, relative deformation area and deformation frequency, n=2. Where the level of value changed is given a range from initial→end value

[a] The maximum possible dA A^{-1} to apply in this technique was 0.09.

[b] This surface dilational viscosity η_d is not under steady state dilation and physically different from the η_d^s in Fig 3.

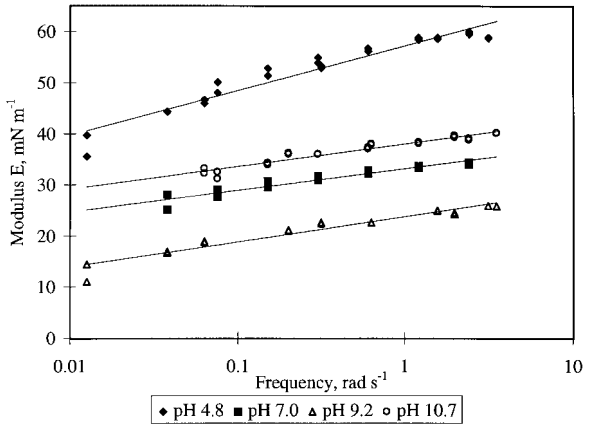

Figure 5. Frequency and pH effect on surface dilational modulus E of egg albumen solution (0.1 g litre^{-1}). Line slopes are: pH 4.8=3.81, pH 7.0=1.85, pH 9.2=2.14, pH 10.7=1.92.

The results of time, relative surface deformation and frequency of deformation for the four different pH values is given in Table 3 and the Figs 4 and 5. The increase at pH 4.8 in modulus E in course of time was an effect of both increasing storage modulus E' and loss modulus E''. As these increases were not proportional it lead to a linear decrease in tan θ, as given in Table 3, indicating a more elastic surface behaviour upon ageing. This could indicate that the protein molecules at this pH interact with each other at the surface layer to build an elastic protein network, and as the time scale of measuring the time effect was of the magnitude 10^3 s, it may be assumed that the network could be build by thiol- and disulphide exchange, which is known to progress slowly at low pH.

This gives the conclusion of a significant increase in the surface dilational modulus in time at pH 4.8, where it reaches the highest value compared to other pH values. The surface has at pH 4.8 a higher tan θ, due to a relatively higher loss modulus than at higher pH values.

As described in the introduction the ability of the surface to stop bubble disproportionation in a foam requires the surface to be completely elastic and the modulus $E > \frac{1}{2}\gamma$. The results in Table 3 show that the surface has significant viscous properties (tan $\theta > 0$), and the loss modulus at pH 4.8 is higher than at pH 7.0–10.7, without significant difference between the last mentioned values. In conclusion of this, the egg albumen surface at any pH does not fulfil the requirements for stopping disproportionation, but a reduction of the drainage velocity is to be expected due to the rigid surface. Foam breakdown consists of other mechanisms too, so these measurements cannot solely predict the foam stability against disproportionation.

Application of an amplitude sweep of the ring movement to create large deformations of the surface enables the linear region to be found, ie the range, where the modulus E is independent of deformation. As given in Table 3, nearly the same linear region was found for all pH values and within ring movements up to 0.1–2.5 mm, which equal relative surface area deformations of 0.0037–0.09, except for pH 4.8, where deformations larger than 0.036 resulted in a drop in the modulus E below 90% of the value at lower deformation rates. Also for pH 4.8, where the deformation of the apparatus could exceed the linear region, the measurements showed no historic effect on the surface, as modulus E values obtained before and after large deformations were similar.

For frequencies smaller than approximately 500 mHz (=3.53 rad s^{-1}) at a dA A^{-1} of 0.037, there was a linear relation between the log frequency and the modulus E. Increasing frequency results in an increase in the modulus E, as presented in Fig 5, and decreasing values of tan θ are seen from Table 3. This property of the surface to present more elastic behaviour at high frequencies, and thereby more viscous at low frequency, is due to the relaxation processes taking place, which take time, and significant relaxation can take place within the time scale of measurements at low frequencies.

Figure 5 shows that both the slope and level of the fitted curve of modulus E for egg albumen surface are higher at pH 4.8, indicating that it is more viscoelastic

	pH			
	4.8	*7.0*	*9.2*	*10.7*
Foamability, relative overrun				
25 s shaking	0.80	0.31	0.17	0.17
60 s stirring	0.35	0.15	0.19	0.09
Foam stability, relative foam volume at 30 min				
25 s shaking	0.404	0.802	0.586	0.643
60 s stirring	0.464	0.583	0.393	0.708
Liquid drainage, ratio drained at 30 min				
25 s shaking	0.765	0.645	0.90	0.764
60 s stirring	0.75	0.50	1.00	0.50
Models of drainage rate (in Fig 6)				
Drainage rate of foam,% liquid $(10^{\alpha}\ \text{min})^{-1}$ (where $\alpha = \log \times \text{min}^{-1}$)	8.4	13.1	12.5	13.8
R^2 for log model	0.9278	0.9836	0.8709	0.9593

Table 4. Foamability, foam stability and liquid drainage from foam of egg albumen protein solution 0.1 g litre^{-1} by two foam formation methods and four pH values

and least purely elastic compared to the other pH values, as the tan θ values given in Table 3 also indicate.

The relaxation processes taking place in the surface include among other mechanisms the exchange of surface-active molecules from the bulk phase to an adsorbed layer, and in a second step unfolding of protein at the surface. On basis of Fig 5 the relaxation at pH 4.8 proceeds in the shortest time, ie goes fast, which correlates to the higher E'' and higher tan θ at this pH, seen in Table 3. At pH 7.0–10.7 relaxation takes place relatively slowly.

From the obtained values of the surface dilational viscosity, $\eta_d = E''\ \omega^{-1}$, a behaviour of the egg albumen surfaces for all pH values like the bulk phenomena 'shear thinning' is seen in Table 3. At pH 4.8 egg albumen had the highest dilational viscosity of the surface.

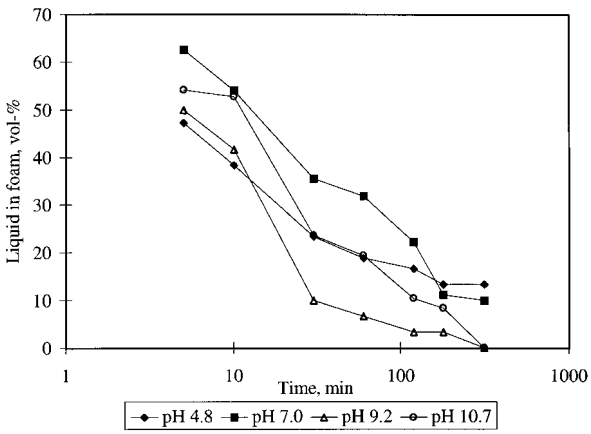

Figure 6. Liquid drainage (% of initial liquid) of egg albumen foams as function of time and pH value. Foams were made by shaking for 25 s.

Measurements of foamability and stability

The foamability by shaking and by stirring of egg albumen solutions is given in Table 4. For both methods the highest overrun is observed at pH 4.8, and the same pH dependency is found for both methods, however, with some differences at pH 7.0 and pH 9.2. In general the overrun by shaking was twice as high as by stirring, except for pH 9.2, where equal overrun values were found. The relatively poorest foam stability was for both foaming methods seen for pH 4.8 and pH 9.2, where less than about 50% of the foam volume remained after 30 min, and foams at pH 7.0 and pH 10.7 were relatively more stable.

The liquid remaining in the foam is shown as a function of time in Fig 6. Here, and in Table 4, it is obvious that drainage has a high rate at pH 9.2, which results in a dry foam remaining after 30 min. The best initial resistance to drainage appears at pH 7.0, but after long periods (>5 h) the foam at pH 4.8, given in Fig 6, has a higher liquid content in the foam and shows a decline in drainage rate in increasing time. All pH values demonstrate that the relative amount of liquid in the foam is a more or less a linear function of the logarithm of the time, although with different rates of drainage. The drainage data in Fig 6 can be fitted into models of $y = -a \log (x) + b$, where the time x (min) and the drainage rate a,% liquid $(10^{\alpha}\ \text{min})^{-1}$, where $\alpha = \log x$, are given in Table 4. This shows an overall slower drainage of foam at pH 4.8 consisting of an initial fast drainage followed by a lower drainage rate.

The results of the measurements by the Free Falling Film Technique showed no light twinkling of the egg albumen in solution, which indicates that spreading particles are not present and coalescence due to this mechanism is not likely. Hole formation was not observed, so hydrophobic particles too are not found to be active under these conditions.

Measurements of bubble size distribution of foam

In Fig 7 examples are shown of bubble size distributions of foam produced by stirring at (a) pH 4.8 and (b) pH 7.0, appearing to obtain the most extreme values of the d_{10} and d_{32} bubble diameters (Table 5). The total protein film surface has been calculated from the obtained foam volume (cm^3) and the measured

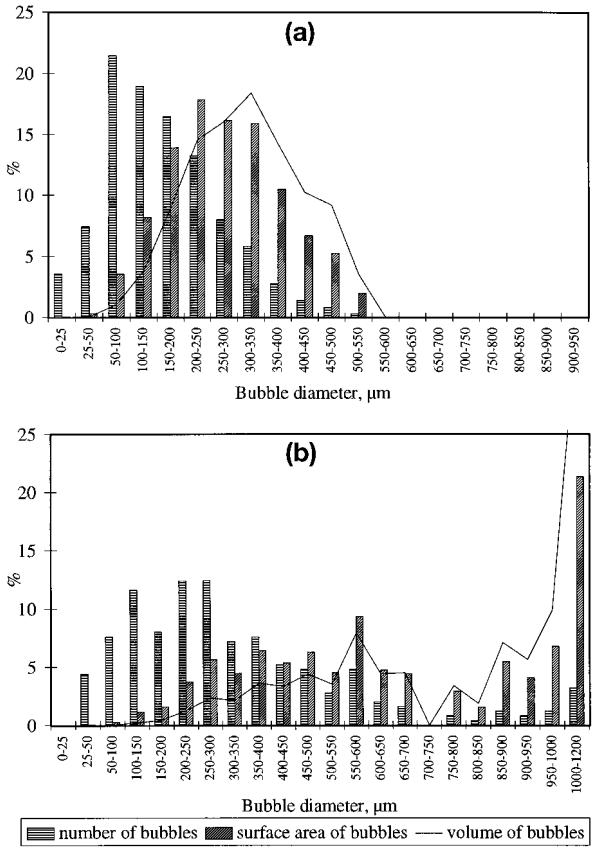

Figure 7. Examples of bubble size distribution (%) of egg albumen foam made by stirring at 2500 rpm for 60 s (a) pH 4.8, $n = 364$, and (b) pH 7.0, $n = 250$.

bubble surface per volume ($\mu m^2 \mu m^{-3}$), and related to protein concentration.

The high foamability and the presence of small bubbles at pH 4.8 correlate well, as the decreasing foamability at higher pH is accompanied by an increase in mean bubble size, although for pH 7.0 very large bubbles dominate the picture, but the corresponding foamability is not poor.

Comparing foam at pH 4.8 and pH 7.0 the foamability differs by a factor 2.3, and the bubble sizes differ by factors 2.3 and 2.5 for the d_{10} and d_{32}, respectively, which means that the relations are within the same order of magnitude. At pH 9.2 and 10.7 both the foam overrun and the bubble mean diameters were very similar, which could be due to only small differences in the protein net charge of egg albumen between these pH values, as the majority of the proteins have a negative net charge at both pH values, and only lysozyme will change from positive net charge at pH 9.2 to zero at pH 10.7. The larger differences between pH 7.0 and pH 9.2, considering the bubble mean diameter, did not resemble the results of foam overrun by stirring, as practically no difference was seen here. There was nearly no difference in the egg albumen protein net charge between these values, which indicates that the foam overrun might reflect this to a higher degree than the bubble size.

DISCUSSION

The results concerning egg albumen show that at pH 4.8, where both positive and negative net charge proteins are present resulting in electrostatic attractions, the influence on surface and foaming properties was significant. This agrees with the review of Mine,[21] where electrostatic interactions are regarded as important for egg albumen's foaming properties. However, protein interactions by intermolecular cross-linking decrease the flexibility and foaming properties,[21] which can be improved by removing intramolecular disulphide bonds from rigid conformation proteins.[22]

The significantly high effect on the foamability of egg albumen at pH 4.8 found here is, however, in contrast with results of Howell and Taylor,[23] who reported that egg albumen at pH 3–3.4 did not expose foamabilities differing from egg albumen at pH 7. The present results concerning foamability and foam stability show that different conditions of the egg albumen protein solution seem to favour (1) foam formation and drainage: the presence of different protein net charges and attractive forces, and (2) foam volume stability: the same or zero net charge of the proteins resulting in repulsive forces.

The present finding of a clear increase in time of the surface modulus E at pH 4.8 compared to higher pH values, where the modulus E exhibited constant levels, can be assumed to be a result of free thiol-disulphide bond exchange, which according to Creighton[9] will take place at slow rates at this pH, or it can be the result of other types of network formation. Doi *et al*,[24] who studied ovalbumin, found that intermolecular disulphide bonds were not essential for the formation of ovalbumin foam, but other non-covalent interactions such as hydrophobic interactions contributed to stable foam formation.

The observations of differences in relaxation of the egg albumen surface at different pH values could be an

Table 5. Mean diameters of bubbles of egg albumen foam at four different pH values, calculated by distribution by number d_{10} and distribution by volume/surface d_{32}, relative standard deviation C_2 and total film surface area of foam, $n = 2$

	pH			
	4.8	*7.0*	*9.2*	*10.7*
d_{10} (μm)	142	328	252	283
d_{32} (μm)	277	694	483	464
C_2	0.47	0.49	0.40	0.37
Width ($d_{32} \pm d_{32} \cdot C_2$ (μm))	147–407	354–1034	290–672	292–635
Total surface ($m^2 g^{-1}$ protein)	76.2	12.9	23.3	11.3

effect of a building up of a protein network, where exchange of molecules at low pH takes longer time and thereby makes the effect of frequency more pronounced. Lucassen-Reynders[25] reports relaxation times from 10^{-3}s for cholesterol/lecithin to 100s for aliphatic alcohols. The present measurements operate with frequencies of $10^{-2} > \omega > 10^{1}$ s^{-1}, where relaxation times within 100s to 10^{-1}s, respectively, can take place, which according to Lucassen-Reynders[25] are realistic values.

As regards the behaviour in the overflowing cylinder of egg albumen solutions, these have, by comparison to pure proteins, a behaviour similar to lysozyme at high film lengths. There is no significant effect on the $d\ln A\ (dt)^{-1}$ in relation to pure water at high film lengths, and at low film lengths egg albumen behaviour resembles β-lactoglobolin giving a 10^{-4} decrease in $d\ln A\ (dt)^{-1}$.[26] This phenomenon, motionless surface, is known for several surface active proteins as a consequence of difficult exchange of molecules from the surface to the bulk contrary to low-molecular surfactants.[27]

One of the most remarkable proteins in egg albumen is ovomucin, as it plays a major role in the viscosity of the thick albumen of native egg white.[28,29] The ovomucin protein is reported to have very high foamability and foam stability, dependent on the size of the molecule, the intrinsic viscosity,[30] and the presence of lysozyme and globulins.[31] Ovomucin is not yet sequenced, its protein structure is unknown, and its interaction with lysozyme at different pH values is unclear. Therefore more work is needed on this subject to evaluate its contribution to egg albumen surface properties and foaming. Sato *et al*[28] refer to results in the '50s' of ovomucin and lysozyme having maximum interaction at pH 7 and decreasing complex formation at increasing pH values. This could explain the relative high foam stability in the present experiments at pH 7.0 compared to pH 9.2, if this interaction participates in the film formation at bubble surfaces. However, the extent of this interaction at low pH values and its role for egg albumen foaming are at the moment unknown.

In conclusion, the foaming behaviour of an aqueous egg albumen solution at pH 4.8, with high overrun, small bubble size and slow drainage of liquid from the foam, can be related to the dynamic surface properties, which are a more rigid behaviour of the surface and a lower dynamic surface tension compared to pH 7.0–10.7.

REFERENCES

1 Wright DJ and Hemmant JW, Foaming properties of protein solutions: comparison of large-scale whipping and conductimetric methods. *J Sci Food Agric* **41**:361–371 (1987).

2 Torikata Y, Kato M, Kumagai H and Yano T, Estimation of foam stability by parameters in the capillary model. *Agric Biol Chem* **55**:1307–1312 (1991).

3 Ferreira M, Behringer R and Jost R, Instrumental method for characterizing protein foams. *J Food Sci* **60**:90–93 (1995).

4 Guillerme C, Loisel W, Bertrand D and Popineau Y, Study of foam stability by video image analysis: Relationship with the quantity of liquid in the foams. *J Texture Stud* **24**:287–302 (1993).

5 Bisperink CGJ, Ronteltap AD and Prins A, Bubble-size distributions in foams. *Adv Coll Interf Sci* **38**:13–32 (1992).

6 Ross S and Nishioka G, Foaming behaviour of partially miscible liquids as related to their phase diagrams, in *Foams*, Ed by Akers RJ, Academic Press, London, UK, pp 17–32 (1976).

7 German JB and Phillips L, Protein interactions in foams: protein–gas phase interactions, in *Protein Functionality in Food Systems*, Ed by Hettiarachchy NS and Ziegler GR. IFT Basic Symposium Series, Chicago, USA, pp 181–208 (1991).

8 Burley RW and Vadehra DV, *The avian egg—chemistry and biology. Wiley-Interscience Publication*, John Wiley & Sons, New York (1989).

9 Creighton TE, *Proteins. Structures and Molecular Properties*, 2nd edn, W H Freeman & Co, New York, USA (1993).

10 Bergink-Martens DJM, Interface Dilation. The Overflowing Cylinder Technique. *PhD thesis*, Agricultural University, Wageningen, The Netherlands (1993).

11 Vadehra DV and Nath KR, Eggs as a source of protein, in *CRC Critical Reviews in Food Technology*, Ed by Furia TE, CIBA-GEIGY Corp, Ardsley, New York, USA, pp 193–309 (1973).

12 Prins A, Principles of foam stability, in *Advances in Food Emulsions and Foams*, Ed by Dickinson E and Stainsby G. Elsevier Applied Science, London, UK, pp 91–121 (1988).

13 Prins A, Dynamic surface tension and dilational interfacial properties, in *New physico-chemical techniques for the Characterization of Complex Food Systems*, Ed by Dickinson E, Blackie Academic & Professional, Glasgow, UK, pp 214–239 (1995).

14 Lucassen J and van den Tempel M, Dynamic measurements of dilational properties of a liquid interface. *Chem Engng* **27**:1283–1291 (1972).

15 Prins A, Dynamic surface properties and foaming behaviour of aqueous surfactant solutions, in: *Foams*, Ed by Akers RJ, Academic Press, London, UK, pp 51–60 (1976).

16 Prins A, Foam stabilizing by milk proteins. *Conference Proceedings: Food Ingredients Europe*, pp 82–85 (1991).

17 van Kalsbeek HK A I, van Aken GA, Bos M and Prins A, Oppervlakte-reologische eigenschappen bepalen vorming en stabiliteit schuim. *Voedingsmiddelen-Technologie* **30**:11–14 (1997) (in Dutch, English summary).

18 Hayakawa S, Kondo H, Nakamura R and Sato Y, Effect of β-ovomucin on the solubility of β-ovomucin and further inspection of the structure of ovomucin complex in thick egg white. *Agric Biol Chem* **47**:815–820 (1983).

19 Kokelaar JJ, Prins A and De Gee M, A new method for measuring the surface dilational modulus of a liquid. *J Coll Interf Sci* **146**:507–511 (1991).

20 Williams A and Prins A, Comparison of the dilational behaviour of adsorbed milk proteins at the air–water and oil–water interfaces. *Colloids and Surfaces* **114**:267–275 (1996).

21 Mine Y, Recent advances in the understanding of egg white protein functionality. *Trends Food Sci Technol* **6**:225–232 (1995).

22 Li-Chan E and Nakai S, Effect of molecular changes (SH group and hydrophobicity) of food proteins on their functionality, in *Food Proteins: Structure and Functional Relationships*, Ed by Kinsella JE and Soucie W, Am Oil Chem Soc, Champaign, IL, USA, pp 232–251 (1989).

23 Howell NK and Taylor C, Effect of ascorbic acid on the foaming and gelling of globular proteins. *Int J Food Sci Technol* **30**:321–334 (1995).

24 Doi E, Kitabatake N, Hatta H and Koseki T, Relationship of SH groups to functionality of ovalbumin, in *Food Proteins: Structure and Functional Relationships*, Ed by Kinsella JE and Soucie W. Am Oil Chem Soc, Champaign, IL, USA, pp 252–266 (1989).

25 Lucassen-Reynders EH, Surface elasticity and viscosity in compression/dilation, in *Anionic Surfactants*, Ed by Lucassen-

Reynders EH. Marcel Dekker, New York, USA, pp 173–216 (1981).
26 van Kalsbeek HKAI and Prins A, Surface properties of proteins in relation to foaming behaviour. *Industrial Proteins* **4**:12–14 (1997).
27 Prins A, Formation and stability of foam made from aqueous protein solutions. *Industrial Proteins* **4**:3–5 (1997).
28 Sato Y, Hayakawa S and Nakamura R, Studies on factors of solubilization of insoluble ovomucin during thick white thinning. *J Agric Food Chem* **24**:798–803 (1976).
29 Hayakawa S and Sato Y, Studies on the dissociation of the soluble ovomucin by sonication. *Agric Biol Chem* **40**:2397–2402 (1976).
30 Kato A, Oda S, Yamanaka Y, Matsudomi N and Kobayashi K, Functional and structural properties of ovomucin. *Agric Biol Chem* **49**:3501–3504 (1985).
31 Johnson TM and Zabik ME, Egg albumen proteins interactions in an angel food cake system. *J Food Sci* **46**:1231–1236 (1981).
32 Hiidenhovi J, Aro HS, Huopalahti R, Suominen I and Kankare V, Studies of egg white proteins by capillary gel electrophoresis, in *Egg and Egg Products Quality, Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products*, Ed by Briz RC. Zaragoza, Spain, pp 319–326 (1995).
33 Stevens L and Duncan D, Peptide mapping of ovoglobulins G2A and G2B in the domestic fowl. *Br Poultry Sci* **29**:665–669 (1988).
34 Stevens L, Egg white proteins. *Comp Biochem Physiol* **100B**:1–9 (1991).