Stable foams with fine bubbles stabilized by the unique protein hydrophobin and its mixtures with milk and egg proteins

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Summary

❖ **HFBII is a class II hydrophobin** isolated from *Trichoderma reesei*. This amphiphilic protein forms the most rigid adsorption layers at the air/water interface among all investigated proteins. HFBII is known also as an excellent foam stabilizer. For this reason, the foam disproportionation (Ostwald ripening) is suppressed.

❖ **Investigated foams from** aqueous solutions of hydrophobin, alone, and from its mixed solutions with other proteins: β-casein, β-lactoglobulin (BLG); ovalbumin (OVA) and bovine serum albumin (BSA). In the case of hydrophobin alone, very stable foams with fine bubbles (R_{32} < 20 μm) were obtained at HFBII concentrations ≥ 0.2 wt%. In the mixed solutions, HFBII can be partially replaced with the globular proteins without any significant decrease of the foam stability. Addition of the disordered protein β-casein to the hydrophobin solutions leads to a pronounced foam destabilizing effect.

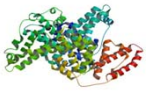
Materials and Methods

Special Protein:

HFBII, 70 amino acids, 7.2 kDa, 4 disulfide bonds

Regular proteins:

β-lactoglobulin (BLG), 162 amino acids, 18.3 kDa, 2 disulfide bonds



bovine serum albumin (BSA), 580 amino acids, 66.4 kDa, 17 disulfide bonds

ovalbumin (OVA), 385 amino acids, 45 kDa, 1 disulfide bond

To produce **foam with fine bubbles** we used a mixer at an appropriate rotation speed, which is a key factor for producing stable foams.

Characterization of the Foams

$$\Phi(t) = \frac{V_F(t) - G_F(t)/\rho_w}{V_F(t)}$$

G_F is weight of the liquid contained in the foam (without the separated serum), $V_F(t)$ is foam volume and $\rho_w \approx 1$ g/cm³ is the mass density of the water phase

Overrun was calculated from the formula:

$$O_{VR}(t) = \frac{\Phi(t)}{1-\Phi(t)}$$

Arithmetic mean radius, R_{10} and volume-to-surface mean radius, R_{32}

$$R_{10} = \frac{\sum_{i=1}^{N} R_i}{N} \qquad R_{32} = \frac{\sum_{i=1}^{N} R_i^3}{\sum_{i=1}^{N} R_i^2}$$

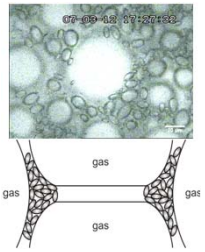
where R_i is the radius of the i-th bubble

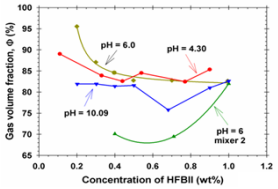
Theoretical *Overrun:*

$$O_{VR} = \frac{w_p \rho_w}{4\varphi_a \rho_p} \frac{R_{3,2}}{a_{3,2}}$$

Protein present as molecules and aggregates of mean volume-to-surface radius a_{32}
w_p the weight fraction , ρ_p is their mass density, $\rho_p \approx 1.35$ g/cm³
R_{32} mean volume-to-surface radius
φ_a is the area fraction, particles at the air/water interface, for close packing of monodisperse spherical particles, $\varphi_a = \pi/ \approx 0.907$

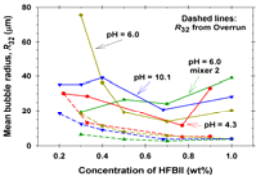
Experimental Results with HFBII



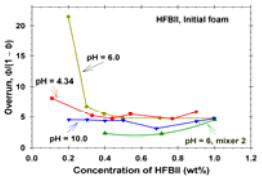


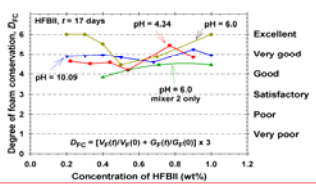
The small bubbles fill and stuck the Plateau borders. (i) block the drainage of water; (ii) increase the air volume fraction; (iii) increase the foam longevity.

Reason for the kinks: In the case of solidifying protein adsorption layers: Optimal agitation time, t_{opt}:
(i) for $t < t_{opt}$ foam creation;
(ii) for $t > t_{opt}$ foam destruction.



Solid lines – R_{32} from the semiautomatic image analysis.
The difference between the two classes of lines is due to the smaller bubbles (< 5 μm) that have not been counted in the semiautomatic image analysis.



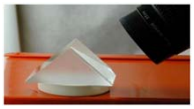


$$D_{FC}(t) = 3 \times \left(\frac{V_F(t)}{V_F(0)} + \frac{G_F(t)}{G_F(0)} \right)$$

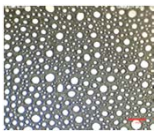
($D_{FC}(t)$ is calculated using the experimental data for $V_F(t)$ and $G_F(t)$: D_{FC} = 6 for an excellent conservation of the foam, i.e. $V_F(t)/V_F(0)$ = 1 and $G_F(t)/G_F(0)$ = 1 (no changes in the foam volume and weight after storage of the foam for a period of time t). The other grades are D_{FC} = 5, 4, 3, and 2, respectively, for very good, good, satisfactory, and poor conservation of the foam.

Experimental Results with Regular Proteins (RP)

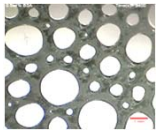
For investigated foam stability with RP we used **Garrett prism method:**



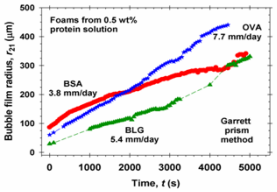
Concentration of RP was fixed at 0.5 wt% and concentration of HFBII was varied in the range from 0.1 to 0.5 wt%.



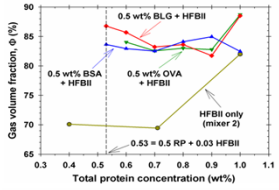
0.5 wt% BSA at o s

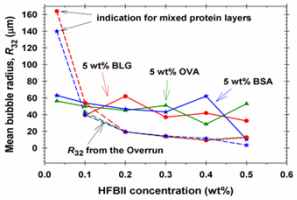


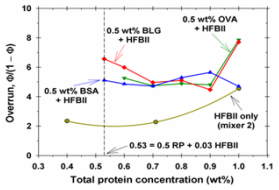
0.5 wt% BSA after 2.6 h



Unstable foams: Decay within 2 – 3 hours.
The mean bubble size is increasing with time.
Microscopic observations: both bubble coalescence and Ostwald ripening take place.







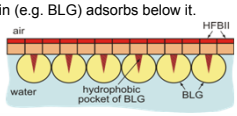
By image analysis of photos 10x we get only the population of big bubbles.

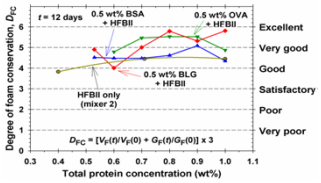
The deviations between the two methods at the lowest C_{HFBII} indicate the formation of a mixed adsorption layer.

The volume fraction of the gas in the foam is Φ = 82 – 89%, and the overrun is 4.5 – 8.
The close values for all regular proteins means that the foaminess is determined (i) by the presence of HFBII and (ii) by the way of stirring (two mixers).
The presence of HFBII is crucial: Below 0.03 wt% HFBII (94:06), no stable foams have been obtained!

All lower HFBII concentrations, a mixed adsorption layer of the two proteins is formed.

At **high HFBII concentrations**, makes a dense layer at the air/water interface and the regular protein (e.g. BLG) adsorbs below it.





HFBII + BLG & OVA at HFBII/RP ≥ 2:7.
All foams are stable for HFBII/RP ≥ 6:94.

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