



Exceptional stability of food foams using class II hydrophobin HFBII

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Abstract

The foam stability of aerated solutions containing the Class II hydrophobin protein HFBII from *Trichoderma reesei* has been investigated and compared with that of other typical food emulsifiers and aerating agents. In simple solutions, we have found that 0.1 wt% HFBII forms exceptionally stable foams across a wide range of solution pH conditions. In aerated solutions comprising xanthan thickener, in order to slow the rate of creaming, we demonstrate that the foams stabilised by HFBII show no significant change in bubble size or air phase volume over a period of at least 4 months. Such foam stability is far in excess of any food-aerating agent of which we are currently aware. HFBII stabilises foams purely by adsorption to the air–water surface, forming a highly elastic surface and providing resistance to both coalescence and disproportionation, without influencing the aqueous phase viscosity. Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.

Keywords: Foam stability; Bubbles; Hydrophobin; Disproportionation; Coalescence

1. Introduction

Liquid foams are thermodynamically unstable and, given time, will eventually coarsen and dissipate (Dickinson, 1992). Liquid food foams generally fall into two categories: those that are created and consumed within a short space of time, so that long-term stability of the foam is not an important consideration (examples include milk shakes, cappuccino, and beer); or those where the rheology of the continuous phase is increased to such an extent that the foam becomes kinetically trapped and effectively stabilised over a substantial period. Examples of this approach include products where the bulk phase is gelled, such as mousse or aerated soft cheese, or partially crystallised, for example ice cream or whipped butter. The ability to produce stable liquid foam, without needing to rely on a gelled or solidified continuous phase, has long been a desire of food manufacturers, but has never been realisable in practice. If this problem could be overcome, and the range of product types that the manufacturer is able to aerate in a stable fashion is broadened, then considerable opportunity would exist to improve product

functionality, create new textures, or reduce the calorific density per volume through aeration.

Aerated foods are subject to destabilisation processes such as coalescence and disproportionation. The latter is a coarsening process (analogous to Ostwald ripening in emulsions) due to diffusion of gas from small bubbles to larger ones, driven by difference in Laplace pressure. In many systems, disproportionation is the most difficult instability mechanism to control (Murray & Ettelaie, 2004). Aside from elevating the continuous phase viscosity, as mentioned above, the most common method of slowing destabilisation processes in food foams is to modify the gas–liquid interface by adsorption of surface-active molecules, such as proteins (Ettelaie, Dickinson, Du, & Murray, 2003; Wilde, 2000). It has been shown that elasticity of the surface is the most important surface property for retarding coarsening by disproportionation. Lucassen (1981) proposed a criterion for the prevention of disproportionation through consideration of the surface dilatational elastic modulus, which, if great enough, can eliminate disproportionation. For a purely elastic surface, this occurs when the following condition is met:

$$E_D = \frac{d\gamma}{d \ln A} > \frac{\gamma}{2} \quad (1)$$

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where, γ is the surface tension, E_D the surface dilatational elastic modulus, and A the surface area.

When measured on an experimental time scale where relaxation can take place, e.g. via diffusional exchange, then E_D is actually a visco-elastic modulus (Bos & van Vliet, 2001; Lucassen-Reynders, Cagna, & Lucassen, 2001), i.e. the surface dilatational modulus. Air–water (a/w) surfaces with adsorbed protein, for example, have a significant viscous response to deformations that are on time scales relevant to disproportionation, i.e. they are not purely elastic in their behaviour. As a result, the condition to prevent disproportionation according to Eq. (1) is never practically met in food products, since a surface-active ingredient that imparts sufficiently high surface dilatational elastic modulus to prevent disproportionation has not yet been identified.

Kloek, van Vliet, and Meinders (2001) provide a theoretical model relating the bulk and surface rheological properties to the process of disproportionation, considering both viscous and elastic contributions. According to their analysis, in order to significantly influence bubble stability over long time scales, a surface elastic modulus of 100 mN m^{-1} or (preferably) greater is required. One of the more elastic of surface-active proteins currently used in food systems is β -lactoglobulin, a globular milk protein that denatures at a surface or interface (Martin, Grolle, Bos, Cohen-Stuart, & van Vliet, 2002). The surface dilatational modulus of this protein has been measured to be of the order of about $30\text{--}40 \text{ mN m}^{-1}$ (Martin et al., 2002; Williams & Prins, 1996) and the surface shear elasticity to be less than 1 mN m^{-1} (Krägel, Wüstneck, Clarke, Wilde, & Miller, 1995; Martin et al., 2002). It can immediately be understood, therefore, that the elastic contribution of β -lactoglobulin, and in fact all other currently used food proteins, falls well short of the level required to inhibit disproportionation based on the criteria set by Lucassen (1981) and Kloek et al. (2001). Experimentally, this is well known and has been readily demonstrated on several occasions: see, for example, Meinders, Bos, Lichtendonk, and van Vliet (2003), Ettalaie et al. (2003), and Dickinson, Ettalaie, Murray, and Du (2002). In each of these reports, adsorbed proteins at the a/w surface in simple solutions could not prevent disproportionation over time scales required for long-term stability in foods products.

Meinders and van Vliet (2004) have further examined the role of the interfacial conditions required to stabilise emulsions. They determined that for typically observed interfacial tensions and dilatational modulus in emulsions, a thick non-desorbing (insoluble) interfacial layer (of the order of the emulsion droplet radius) was required to prevent disproportionation. Similar requirements would be required for stabilisation of bubble dispersions and foams. However, such a surface structure is not produced using common food proteins and, therefore, long-term stability of such foams is not observed.

There has been an increasing amount of activity relating to stabilisation of foams. In the context of foods, in

particular, a review of recent developments of foam stabilisation has been published by Murray (2007). We have recently reported (Cox, Cagnol, Izzard, & Russell, 2007) on the remarkable surface activity of hydrophobin proteins derived from the filamentous fungus *Trichoderma reesei*, and the influence these have on the stability of some small-scale bubble dispersions. Hydrophobins are a family of small proteins ($7\text{--}15 \text{ kg mol}^{-1}$) first identified in *Schizophyllum commune* (Wessels, de Vries, Asgeirsdottir, & Springer, 1991) and subsequently isolated from a number of filamentous fungi (Linder, Szilvay, Nakari-Setälä, & Penttilä, 2005). Two classes of hydrophobin have been distinguished based on hydropathy profiles (Wessels, 1994), a measure of the hydrophobicity profile of protein (Kyte & Doolittle, 1982), and aqueous solubilities (Wessels, 1994): Class I hydrophobins (e.g. SC3 from *S. commune*) form highly insoluble aggregates which can only be dissolved with strong acids (de Vocht et al., 1998). Class II hydrophobins (such as the HFBI and HFBII hydrophobins from *T. reesei*) are more readily solubilised, and can be readily dissolved in aqueous solution. In our previous paper (Cox et al., 2007) we reported measured a/w surface shear elastic moduli for HFBI and HFBII hydrophobins, which were in the range $500\text{--}700 \text{ mN m}^{-1}$. These values represent more than an order of magnitude increase over moduli of other commonly used food proteins. While the condition for elimination of disproportionation may not be strictly met by this degree of elasticity, since our data was a measure of shear as opposed to dilatational elasticity, it is probable that it can be sufficiently retarded to allow control of foam over useful product shelf-lives. Indeed, we then showed how the high surface elasticity of HFBII could stabilise single bubbles to disproportionation with atmospheric air for significant periods of time, when compared to both β -casein and β -lactoglobulin, and also that HFBII could form a stable bubble dispersion for at least 4 days at room temperature.

These data highlight the potential of hydrophobins to form stable bubbles and foams by surface adsorption alone. The ability to stabilise foam through surface adsorption as opposed to stabilisation via the bulk phase could provide the real potential to formulate aerated products, which could be designed with the appropriate continuous phase around a stable air phase.

In this current study we apply the use of hydrophobin to foams, in both model systems and an example food product, comparing them with some other typical food emulsifiers/aerating agents. We examine the extent to which foam stabilisation can be achieved and discuss the potential we see for hydrophobins as novel foam-stabilising ingredients, particularly in the context of food products.

2. Materials and methods

2.1. Materials

Class II hydrophobin (HFBII) was obtained from VTT Biotechnology (Espoo, Finland), having been prepared as

previously described (Bailey et al., 2002; Linder et al., 2001). Briefly, the hydrophobin protein was made by fermentation of a *T. reesei* culture, extracted, and subsequently purified. In this case, the hydrophobin was provided by VTT Biotechnology in an ammonium acetate buffer solution. In order to remove much of this, the aqueous solution was freeze dried to remove water and buffer and then stored at 40 °C in a vacuum oven (Gallenkamp) for 18 h. Removal of most of the buffer in this way aided preparation of solutions at different pH, most notably at pH greater than ca. 7. The hydrophobin protein was then reconstituted in pure water to a known concentration (ca. 0.5 wt%) and stored frozen. β -Lactoglobulin (from Bovine Milk, ca. 90%), Tween 80, and sodium azide were purchased from Sigma-Aldrich. Citric acid (99.5%+) and sodium hydroxide (97.5%) were obtained from BDH and Fisher Chemicals, respectively. Sodium caseinate (Na Cas >90% protein content) was purchased from DMV International. Xanthan (cold water dispersible, Keltrol RD) was purchased from CP Kelco. Sucrose was obtained from Tate and Lyle, UK, skim milk powder from Dairy Crest, UK (ca. 35% protein content), and Hygel 8293 (hydrolysed milk protein, produced by enzymatic solubilisation, 80% minimum protein content) from Kerry Bio-sciences, UK. Green and Black's organic hot chocolate powder contained 28.5 wt% cocoa powder, 16.3 wt% dark chocolate, total protein 9.1 wt%, carbohydrate 63.5 wt%, and fat 9.3 wt%, and was produced by Green and Blacks Ltd., UK. Aqueous solutions were prepared using distilled water (Elga Water purifier, surface tension 72 mN m⁻¹ at 20 °C). Apart from the HFBII, as discussed above, all ingredients were used as received without further purification.

2.2. Solution preparation and aeration

Mix preparation: Aqueous solutions containing protein or surfactant were made at the appropriate concentration in water or in water containing 0.4 wt% xanthan gum, as a thickening agent (also referred to as continuous phase stabiliser). A summary of all the formulations used is shown in Table 1. For the solutions to be made up in water without xanthan gum (Formulations 1a–8a), Na Cas, Hygel, β -lactoglobulin, and Tween 80 were mixed for approximately 30 min in water to ensure complete dispersal. For the case of HFBII, this was added as an aliquot of concentrated solution into water. The concentrated stock solution was sonicated for 20 s immediately prior to dilution in order to dissolve any weak macroscopic aggregates that can form through shearing solutions of hydrophobin, as discussed previously (Cox et al., 2007). For solutions prepared in water with 0.4 wt% xanthan gum (Formulations 1b–8b), Na Cas, Hygel, and β -lactoglobulin powder were mixed with xanthan powder before dispersing in water. For solutions prepared with either Tween 80 or HFBII, these ingredients were added to the aqueous phase after xanthan gum had been fully dispersed in water. All

Table 1
Formulation details for each of the foams prepared

Surface active agent	Concentration (wt%)	Formulation, no xanthan	Formulation with 0.4 wt% xanthan
Sodium caseinate (Na Cas)	0.25	1a	1b
β -Lactoglobulin (β -LG)	0.25	2a	2b
Hygel	0.25	3a	3b
Tween 80	0.25	4a	4b
HFBII	0.1	5a [3.6]	5b [4.0]
HFBII	0.1	6a [5.5]	6b [5.5]
HFBII	0.1	7a [7.2]	7b [7.4]
HFBII	0.1	8a [8.4]	8b [8.6]

Formulations 1a–8a contain aerating agent in water. Formulations 1b–8b contain aerating agent in water plus 0.4 wt% xanthan gum. Numbers in brackets represent the measured solution pH.

solutions containing xanthan were mixed at room temperature for at least 1 h to ensure good dispersal and hydration of this polysaccharide. Na Cas, β -lactoglobulin, and Tween 80 were all made up in water, and no change in pH was made. HFBII solutions were prepared across a range of pH conditions, and the pH was controlled by addition of either citric acid or sodium hydroxide. All solutions had 0.05% sodium azide added as a preservative, post-aeration.

Aeration: 75 mL of aqueous solution, with dissolved protein or surfactant, was aerated to ca. 150 mL using an aerolatte (Radlett, UK) hand held electric mixer at room temperature. The device consists of a whisk rotor, which is a wire coil shaped in a horizontal circle with an outer diameter of 22 mm rotated about a vertical axis through its centre at a rotational speed of approximately 12,000 rpm in air. Formulations 1a–8a (without stabiliser) were mixed and aerated for 2 min in order to ensure that both the appropriate air phase volume was incorporated and thorough bubble break-up were achieved. The measured air phase volume fraction at time $t = 0$ was within the range 0.5–0.6. Formulations 1b–8b (containing stabiliser) were mixed and aerated for 4 min. This time scale was longer than for the formulations with no thickener, since the addition of xanthan led to slower incorporation of the required volume of air using this aeration device. This process resulted in a foam of measured air phase volume fraction ca. 0.5 at time $t = 0$. Subsequently, portions of the prepared foam were portioned out for subsequent stability tests, as described below. All foams were stored at 5 °C.

2.3. Foam and bubble stability analysis

Analysis of foam stability through measurements of bubble size and foam volume was determined via the following experimental methods:

(i) *Total air phase volume as a function of time*: 100 mL of foam was poured into a measuring cylinder. Changes in the foam height F_h were measured as a function of time. We calculate the *total air phase volume fraction* ϕ_{air} in the measuring cylinder using

$$\phi_{\text{air}} = (F_h - V_w)/F_h \quad (2)$$

where V_w is the total amount of aqueous phase in the foam. Once the foam has collapsed, $F_h = V_w$ and $\phi_{\text{air}} = 0$. The value for V_w was noted for each sample, typically being ca. 50 mL.

Formulations 1a–8a did not contain any thickening agent and, as a result, the bubbles rapidly creamed to the surface. This leads, within several minutes, to an upper phase rich in air bubbles (a high air phase volume part) and a lower phase depleted of air bubbles (a low air phase volume part). The calculation of *total air phase volume* using Eq. (2) does not take into account such system heterogeneity. Hence, by ϕ_{air} we mean total air phase volume within the 100 mL measuring volume of the cylinder. Therefore, this allows comparison between mixes where the foam creams quickly (Formulations 1a–8a) and mixes where creaming is slowed down due to the presence of a thickener (Formulations 1b–8b).

(ii) *Air bubble size as a function of time measured using light scattering*: 20 mL of foam was analysed using a Turbiscan (TLAb Expert, Fullbrook Systems Ltd., UK). This instrument measures transmitted (180° from the incident) and backscattered (45° from the incident) light from a cylindrical sample vial containing the foam. In simple aerated formulations where air bubbles are the principal scattering species, bubble size changes can be monitored through variations in the backscattered light through the central area of the sample vial. The backscattered level BS is related to the photon transport mean free path λ^* through the foam via

$$\text{BS} = \frac{1}{(\lambda^*)^{1/2}} \quad (3)$$

λ^* is dependent on the gas phase volume fraction ϕ_{air} and the bubble mean diameter d through

$$\lambda^* = \frac{2d}{3\phi_{\text{air}}(1-g)Q} \quad (4)$$

where Q is the scattering efficiency factor and g is an asymmetry factor. For the purposes of these studies, the bubble size was determined between the heights of 20 and 30 mm above the base of the sample vial. This region of the foam was chosen such that measurement of bubble size could be made for the longest period of time without the complication of foam collapse and significant creaming in this area of the foam. Hence, data were only taken to the time point where foam collapse or the serum level reached this region of the vial, or to the point where the air phase volume fraction in this region deviated significantly from 0.5.

(iii) *Air bubble size as a function of time observed using optical light microscopy*: This method was used to visually compare the stability of foams produced using Formulations 1b and 6b over a period of up to 1 week at 20°C . Foam was carefully placed into the well of a Coverwell Press-Seal imaging chamber (size 20 mm diameter \times 2.5 mm depth, Sigma-Aldrich) and then a microscope glass slide was pressed and fixed to this. This sealed set-up allowed visual observation of the foam over several days without evaporation of the aqueous phase. A depth of 2.5 mm was found most appropriate since no significant distortion of air bubbles was observed to result when attaching the chamber to the glass slide. Furthermore, significant air bubble growth could occur without subsequent distortion of the air bubbles. Foams were visualised using a LeicaDMR microscope, using a $\times 10$ magnification objective. The microscope was attached to a JVC KY-F75U3CCD camera and connected to a computer through a FireWire connection. Images were digitally captured using KY-Link software (Optivision, Leeds, UK).

2.4. Measurement of continuous phase rheology

Continuous phase moduli for elasticity (G'), viscosity (G''), and apparent yield stress were measured for a 0.4 wt% aqueous solution of xanthan gum at 5°C , using an AR-G2 rheometer (TA Instruments Ltd., Crawley, UK) with a cone and plate geometry, where the stainless-steel cone was 6 cm diameter with a 2° angle. G' and G'' were measured across a frequency range of 0.1–100 Hz, subjecting the solution to an oscillatory amplitude of 5×10^{-3} rad. The apparent yield stress was determined using a stepped flow approach to measure viscosity as a function of shear stress and shear rate. To calculate the apparent yield stress, the shear-thinning regime of the data was fitted to a Hershel–Bulkley model.

2.5. Measurement of surface shear rheology

The surface shear elastic modulus, $G'(s)$, and viscous modulus, $G''(s)$ were measured for the a/w surface of a solution of 0.01 wt% HFBII in water using an AR-G2 rheometer. A Pt–Ir Du Noüy ring set-up was used (13 mm diameter ring, Krüss, Germany, flamed prior to use) with a glass dish of 60 mm diameter. $G'(s)$ and $G''(s)$ were determined at 20°C as a function of oscillation frequency (between 0.001 and 1 Hz) using a constant oscillation amplitude of 5×10^{-3} rad. The solution of hydrophobin was sonicated in an ultrasound bath for 20 s before loading on to the rheometer. Once the ring was in position at the a/w surface, the sample was then allowed to equilibrate for 1 h before measurement was started. This ageing time was used in order to allow adsorption of HFBII to the surface, i.e. reach equilibrium.

2.6. Preparation of chocolate milk shake

An aerated chocolate milk shake, stabilised using hydrophobin was prepared to the following overall formulation: 10 wt% skim milk powder (ca. 35 wt% protein content), 8 wt% chocolate powder, 0.4 wt% xanthan gum, 1 wt% sucrose, 0.1 wt% HFBII, and 80.5 wt% water. Two separate mixes (A and B) were prepared, which when blended gave the overall formulation. Mix A was 35 g of an aqueous solution of HFBII, where the concentration of hydrophobin was 7.2 mg mL^{-1} . Mix B consisted of the remaining ingredients, which were blended together and mixed with water, totalling 100 g. This mix was then heated to 80°C for 30 s, before cooling to 5°C . Mix A was then aerated using an aerolatte to a volume of 120 mL. The foam was then gently mixed with Mix B, and the air phase volume measured to be ca. 0.4. 100 mL of aerated chocolate milk shake was then poured into plastic bottles, 0.05 g sodium azide was added, and then stored at 5°C . Bubble stability was assessed visually over a period of 3 weeks.

3. Results and discussion

3.1. Foam stability in aqueous solution with no added stabiliser

Aqueous solutions aerated without the addition of a thickening agent rapidly separate into two distinct layers consisting of an upper foam-rich phase and a lower aqueous-rich phase. This is due to drainage of the liquid and creaming of the bubbles. For the solutions aerated in this work, initially the air phase volume was ca. 0.5, i.e. below the close-packed limit for spheres of equal size. However, as the bubbles quickly rise to the surface, the air phase volume in the upper part of the foam phase increases rapidly. Ultimately, this leads to close packing of air bubbles and, subsequently, a polyhedral foam. Therefore, these experiments are a measure of the stability of bubbles in a “dry” foam environment.

Total air phase volume as a function of time is plotted in Fig. 1, for Formulations 1a–8a. The milk protein stabilised foams collapse rapidly, with complete loss of air within less than 24 h. The foam stabilised by Tween 80 shows greater air phase stability than the milk proteins since this surfactant is able to stabilise a polyhedral foam structure more. Nevertheless, each of these typical food emulsifiers/aerating agents forms foams in water with far shorter lifetime than those stabilised by HFBII. At each of the four solution pHs studied, HFBII forms a foam that is stable to any significant air phase loss ($>10\%$) over a period of at least 5 days. Subsequent collapse and air phase loss is then relatively gradual, and some foam is present for at least two or more weeks. Clearly, HFBII can form highly stable foams in such conditions where a polyhedral foam structure will be formed and, furthermore, such stability is observed across a wide range of pH conditions.

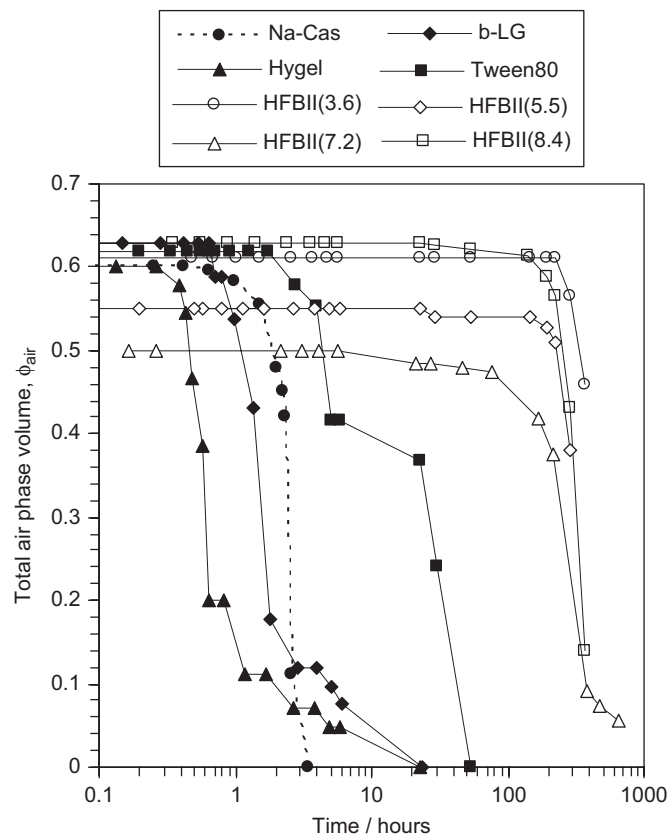


Fig. 1. Effect of aerating agent type on foam stability, expressed as total air phase volume as a function of time, for Formulations 1a–8a.

3.2. Foam stability in aqueous solution with added stabiliser: reduced creaming rate

Addition of xanthan gum has a significant effect on the rate of creaming, due to the apparent yield stress, which this polysaccharide provides to the continuous phase. Initially, this effectively traps the bubbles in position since the yield stress opposes the buoyancy force. However, as the foam starts to destabilise, through the mechanisms of coalescence and disproportionation, the bubbles start to grow. Ultimately, the size of the bubbles will lead to a buoyancy force, which exceeds that of the yield stress, and creaming will occur. Such conditions have been described by Dutta et al. (2004). We chose a concentration of xanthan such that creaming would be largely prevented until significant bubble growth had occurred; therefore, the results in this section provide a clear indication of bubble stability at around or below the close-packed limit. In this regime, one would expect that disproportionation would be the dominant destabilising mechanism since relatively fewer bubble would be in very close contact. As bubbles grow, however, some creaming occurs, the effective volume fraction in the upper part of the measuring cylinder increases, and coalescence would start to become a more significant factor. Another reason for adding xanthan to slow the rate of creaming was that the dispersed nature of the air phase in such a foam can be viewed as being more

representative of that in many aerated foods such as whipped cream, ice cream, and mousse.

Total air phase volume as a function of time for each of the Formulations 1b–8b is shown in Fig. 2. In terms of foam stability, addition of xanthan clearly leads to more stable, persistent foams. This is basically as one would expect, because xanthan provides a degree of stability via continuous phase gelation, restricting bubble movement and therefore slowing the creaming and coalescence rates.

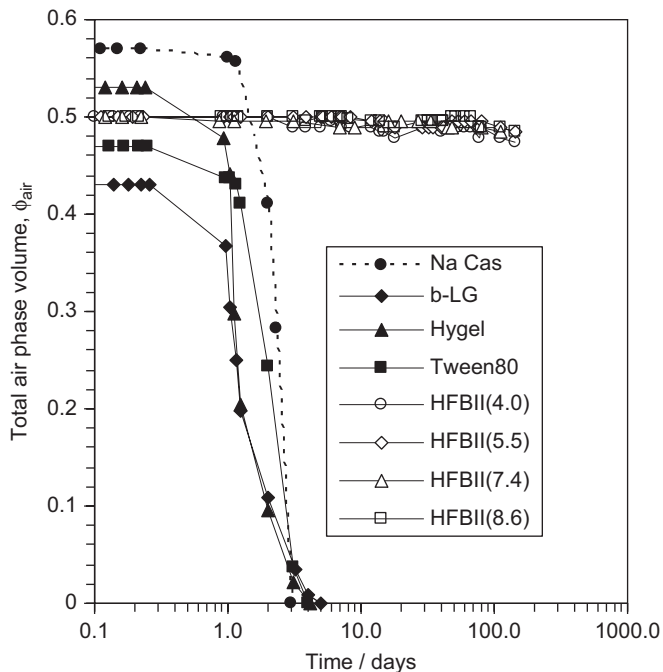


Fig. 2. Effect of aerating agent type on foam stability, expressed as total air phase volume as a function of time, for Formulations 1b–8b, where the continuous phase contains 0.4wt% xanthan gum.

Bulk elasticity is also understood to retard the rate of disproportionation (Kloek et al., 2001), although it is unlikely to have a notable effect here. Our bulk rheological measurements of a 0.4 wt% solution of xanthan (containing no protein) are shown in Fig. 3a, where the influence of oscillation frequency on the elastic and viscous moduli is plotted. Since the rate of disproportionation (and therefore rate of bubble compression/expansion) is a variable dependent on bubble size, the elastic nature of the continuous phase needs to be significant across a range of deformation rates, which we measure via the frequency sweep. The values for G' shown in Fig. 3a are at least an order of magnitude lower than those required to have any notable impact on disproportionation, based on theoretical work by Kloek et al. (2001). The value of G' also decreases as the frequency of oscillation decreases, i.e. for smaller bubbles, where the rate of disproportionation is the greatest; there is a lesser influence of the continuous phase elasticity. Therefore, we can be confident that the principal stabiliser to disproportionation in each foam will be the surface adsorbed layer, i.e. the nature of the a/w surface due to the adsorption of protein or surfactant.

Indeed, this is what we observe: β -Lactoglobulin, Na Cas, Hygel, and Tween 80, all stored at 5 °C produce foams, where the bubbles grow and the foam collapses completely within 5 days. In fact, >75% of the foam volume has been completely lost within about 24–48 h for these formulations, where over this time period each of the foams shows significant bubble growth and foam collapse.

By some considerable margin, the most stable foams are those formed using hydrophobin HFBII, both in terms of retaining the foam volume and in maintaining bubble size. In fact, at all four pH conditions measured, HFBII shows similar foam volume stability (via surface adsorption), and this stability is far in excess of any food protein or

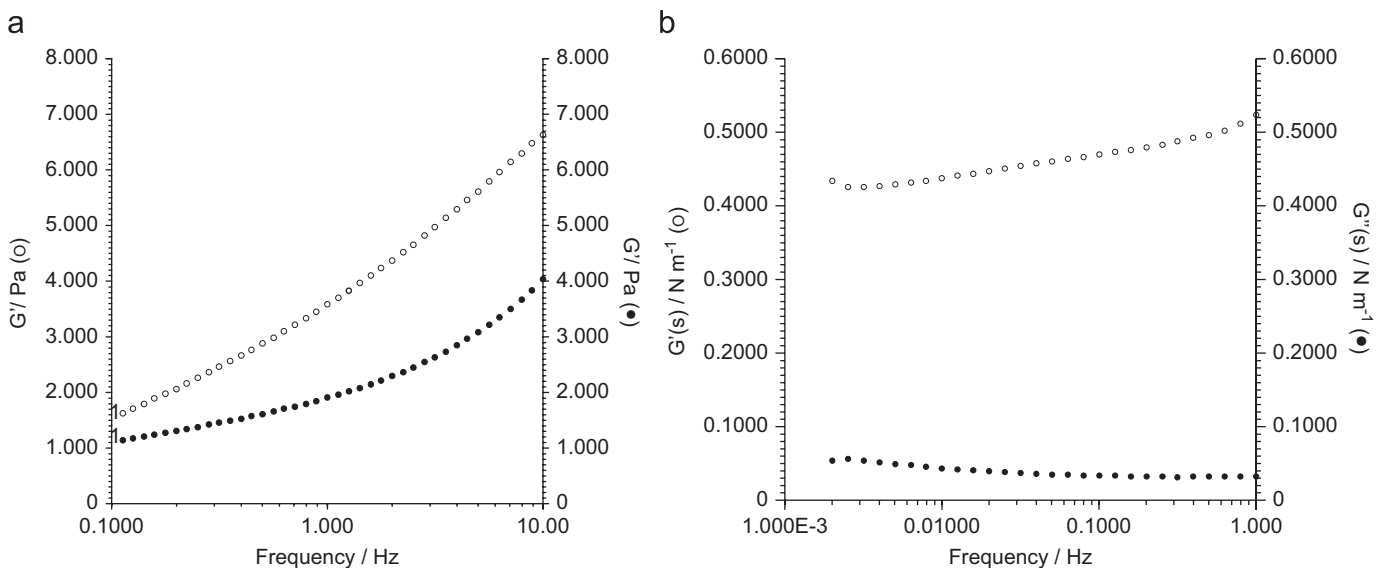


Fig. 3. (a) Continuous phase elastic (G') and viscous (G'') moduli measured as a function of oscillation frequency for a solution of 0.4 wt% xanthan gum. (b) Surface shear elastic ($G''(s)$) and viscous ($G'(s)$) moduli measured as a function of oscillation frequency at an a/w surface of a solution of 0.01 wt% hydrophobin HFBII.

surfactant that we are aware of. Fig. 3b further shows the effect of oscillation frequency on both the surface shear elastic and bulk moduli of the a/w surface stabilised by 0.01 wt% HFBII at pH 5.5. Although there is a general trend towards a lower $G'(s)$ at low frequencies, it is quite apparent that the surface retains its highly elastic nature across the range of frequencies tested. One therefore concludes that the mechanical properties of the a/w surface formed by the adsorption of HFBII protein are directly responsible for foam stability.

At the extremes of pH (4.0 and 8.6), the foams stabilised by HFBII do start to show some gradual creaming over a period of about 5 days, although this is a slow process and the interface between the serum phase and the foam phase was not well defined, i.e. although there was some depletion of bubbles in the lower serum phase, there was still a significant number of small bubbles located there. This process is likely to be due to some larger bubbles being formed during the aeration stage, which were too buoyant for the yield stress to prevent from creaming, as opposed to significant bubble instability and growth via disproportionation and coalescence. Nevertheless, a more detailed study of pH effects on bubble stability would be useful to determine any differences in the stabilisation capability of hydrophobin across a range of solution conditions. That said, HFBII clearly has considerable foam-stabilising capability from pH 4.0 to 8.6. This is consistent with previously measured surface shear rheology data (Cox et al., 2007), which shows high values of elastic modulus ($> \text{ca. } 0.3 \text{ N m}^{-1}$) across a wide range of pH units (from pH 3 to 9).

We demonstrate further the long-term foam stabilisation capability of HFBII in a solution containing thickening agent. Fig. 4 shows two foams: one stabilised using 0.1 wt% HFBII and the other stabilised using 0.5 wt% Na Cas. In both examples, the aqueous solution contained

0.5 wt% xanthan. At time = 0, the measuring cylinders were filled to 100 mL of foam, consisting of $\phi_{\text{air}} = 0.5$. Within 1 week, the foam stabilised by Na Cas had completely collapsed. However, in the case of the HFBII stabilised foam, over a period of 2 years and 5 months, less than 20% of the air phase volume had been lost. A proportion of this volume loss can probably be attributed to evaporation of water. Furthermore, although clearly some creaming has taken place over this considerable time period, visibly the air bubble size barely changed.

3.3. Bubble size as a function of time measured by light scattering

The growth in bubble size over time was measured for each of the Formulations 1b–8b as a function of time, using the light scattering technique described. Figs. 5a and b show air bubble diameter d_t relative to that at time $t = 0$ for each of the foams; d_t is calculated as a function of time in terms of d_0 , the diameter at $t = 0$, and d_t , the diameter at time t , as

$$d_r = \frac{d_t}{d_0} \quad (5)$$

The sizes of air bubbles at $t = 0$ are also summarised in Table 2, where $t = 0$ is the first measurement taken within 1 min of foam formation. In Fig. 5a it can be seen that each of the dairy based proteins and Tween 80 clearly show rapid bubble growth over a period of 1 day, growing to about three times their initial diameter; the measurement of size was then stopped. This increase in size leads to creaming of the air phase and foam collapse, as already discussed. Each of the foams stabilised by HFBII, however, shows very little change in air bubble size over a period of 4 months. Formulation 5b (HFBII at pH4.0) actually shows what appears to be a bubble size decrease over time. We

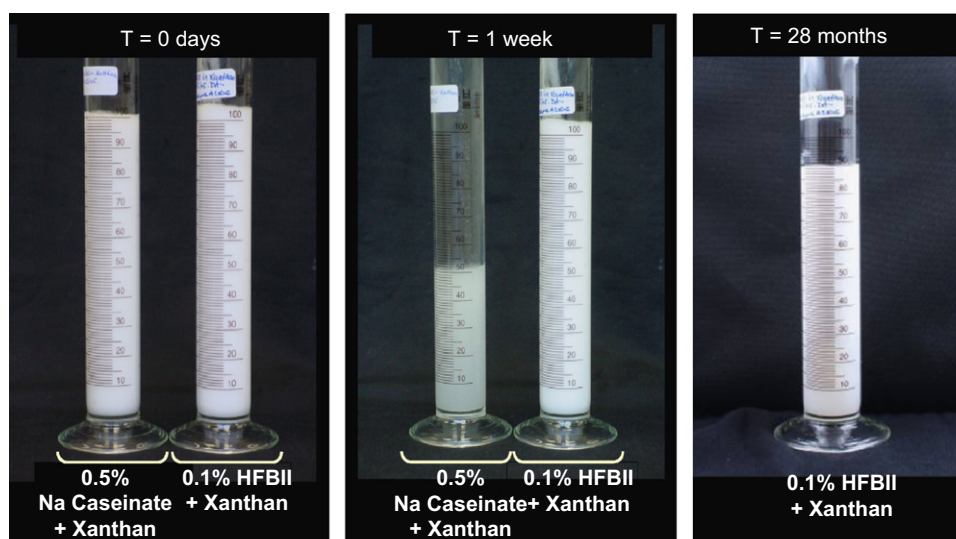


Fig. 4. Photograph of foams containing 0.5 wt% sodium caseinate and 0.1 wt% HFBII, both with 0.5 wt% xanthan gum after different storage periods at 5°C. Sodium azide was added to prevent microbial growth.

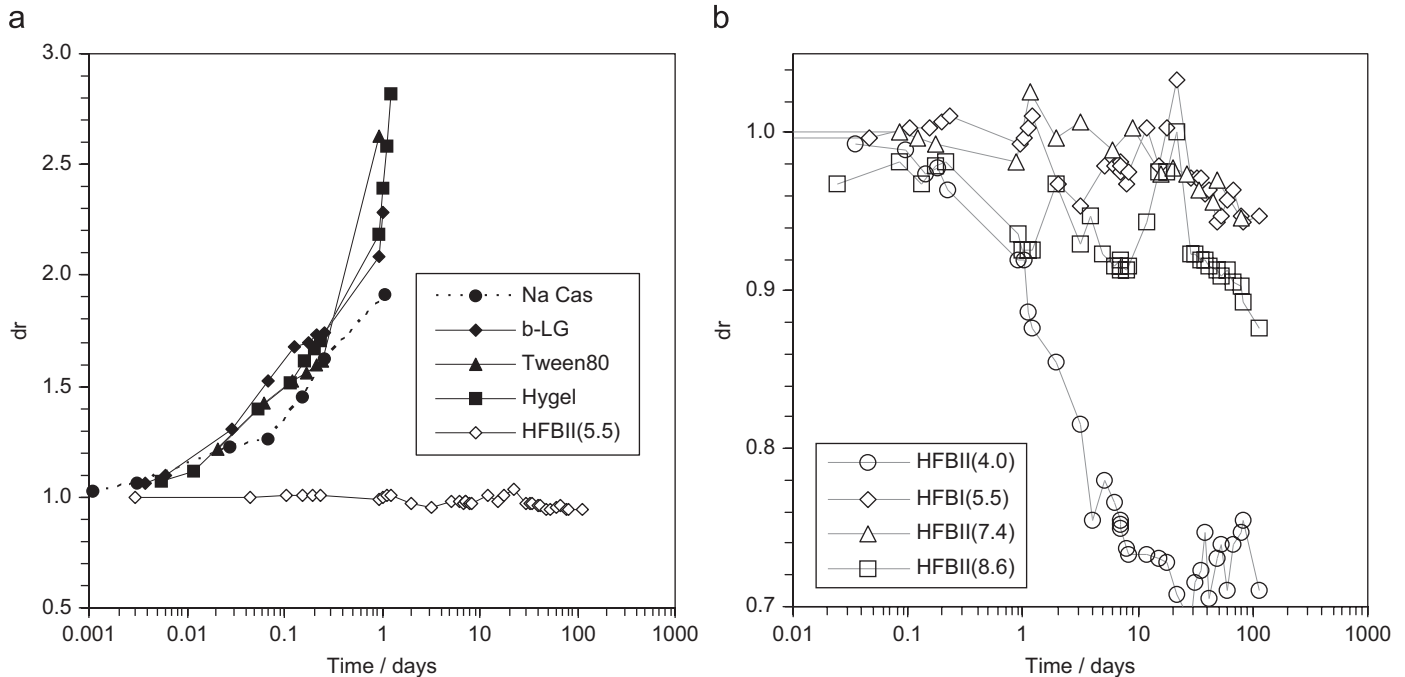


Fig. 5. (a) Change in air bubble size as a function of time for sodium caseinate, β -lactoglobulin, Hygel, Tween 80, and HFBII at pH 5.5. (b) Change in air bubble size as a function of time for HFBII at four different pH conditions. The continuous phase of each of the foams contained 0.4 wt% xanthan gum.

Table 2
Initial mean air bubble diameter for each of the Formulations 1b–8b, containing xanthan thickener

Formulation	Mean initial bubble diameter (μm)
Sodium caseinate	194
β -Lactoglobulin	206
Hygel	185
Tween 80	193
HFBII (3.6)	236
HFBII (5.5)	132
HFBII (7.4)	114
HFBII (8.6)	132

believe that the overall size distribution is not actually changing to a great extent and, visually we noted no significant coarsening of the foam throughout the vial throughout the experiment. Furthermore, there is no collapse or air loss of the foam throughout this period, as is clear from Fig. 2. However, since the initial air bubbles formed in this formulation exhibited a larger size than the other hydrophobin foams (as indicated in Table 2), one is probably measuring the effect of some creaming in the vial over the measuring area of 20–30 mm height. As a result, some of the larger bubbles cream beyond the measurement area, leaving a larger proportion of smaller bubbles. Almost all of this change occurs within about 5 days, and from then onwards the size is constant.

Another interesting point to note from the initial bubble size data is that with the exception of the HFBII foam formed at low pH (4.0), the foams stabilised by HFBII have significantly smaller bubbles than the other foams

measured. These data indicate that the surface activity of HFBII facilitates effective bubble break-up, and this is reinforced by the elastic nature of the bubble surface, preventing coalescence and disproportionation from an early stage.

3.4. Optical light microscopy study of foam stability

Stability of Formulations 1b (Na Cas) and 6b (HFBII at pH 5.5) were compared using optical microscopy to observe the evolution of bubble size over several days. Fig. 6 shows images of these foams at three different time points. These image sequences were captured from a time-lapse video, which can be downloaded electronically from the “supplementary information” section.

The foam stabilised by Na Cas shows rapid bubble growth over 12 h. In contrast, the foam stabilised using HFBII shows very little change in bubble size over a period of 6 days. We also draw attention to the presence of some non-spherical bubbles, which we have highlighted previously (Cox et al., 2007), where we believe the high surface elasticity is great enough to prevent the bubble reverting to spherical geometry through surface tension forces. These images visually demonstrate the effectiveness of hydrophobin HFBII as a foam stabiliser, dramatically reducing both coalescence and disproportionation between air bubbles over time periods far in excess of other food proteins.

A calculation of the Laplace pressure can be obtained from the bubble size and the surface tension. We have previously measured the equilibrium surface tension of HFBII to reach 30 mN m^{-1} (Cox et al., 2007). For the case

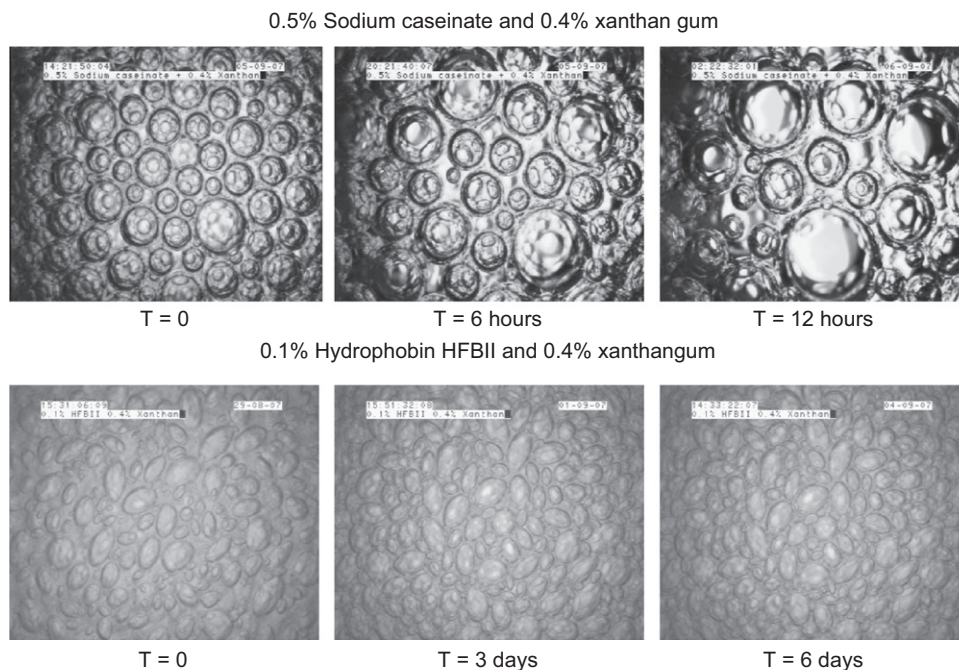


Fig. 6. Images of foams captured at different time points, stabilised by sodium caseinate (Formulation 1b, above) and hydrophobin HFBII (Formulation 6b, below). Magnification $\times 10$.

of a bubble of radius $50\ \mu\text{m}$ stabilised by HFBII, the Laplace pressure is $1200\ \text{Pa}$. Although this value is significantly smaller than that calculated for a similar bubble stabilised by, for example, β -lactoglobulin ($2000\ \text{Pa}$ for a similar size bubble with surface tension $50\ \text{mN m}^{-1}$), the magnitude still provides a driving force for bubble dissolution. This means that the protein must exhibit some resistance to compression and bending of the adsorbed layer.

3.5. Foam stability in complex formulations

Foams stabilised by surface adsorption of HFBII clearly show stability well in excess of other food surfactants in simple aerated solutions. Here, we further highlight the effectiveness of hydrophobin as a foam stabiliser in a more complex formulation, i.e. a chocolate milk shake (Fig. 7). Although the formulation contains a number of further ingredients (cocoa powder, fat, protein, sugar), the foam phase shows good stability for a period of at least 5 weeks when stored at $5\ ^\circ\text{C}$, i.e. there has been no apparent loss of air phase volume and there has been no visible increase in air bubble size. HFBII can reduce the a/w surface tension to values below that of the other surface-active agents present. For example, the a/w equilibrium surface tension of skim milk protein has been measured to be $48\ \text{mN m}^{-1}$ (Rouimi, Schorsch, Valentini, & Vaslin, 2005), compared to a value of $30\ \text{mN m}^{-1}$ obtained for HFBII (Cox et al., 2007). Furthermore, HFBII also forms a highly elastic surface film. Consequently, once adsorbed at the bubble surface, the addition of other ingredients do not appear to

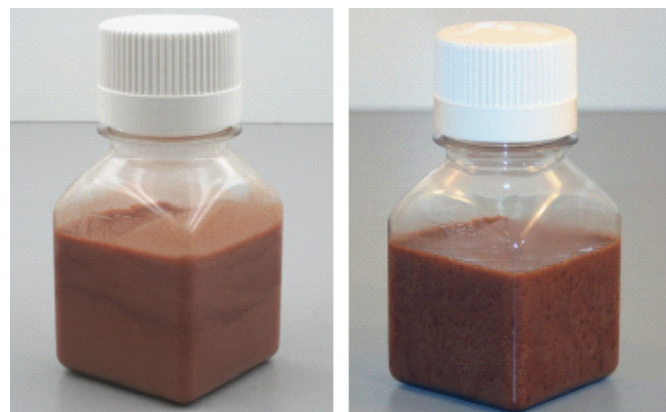


Fig. 7. Chocolate milk shakes prepared using $0.1\ \text{wt}\%$ HFBII. Product at $t = 0$ (left) and after 5 weeks storage at $5\ ^\circ\text{C}$ (right). The air phase volume fraction was 0.4 . The product stored for 5 weeks appears to have some structural heterogeneity when compared to that at $t = 0$. This is not visibly due to a change in bubble size, but appears to be due to movement of material within the sample, notably the dark chocolate particles.

greatly inhibit the surface-stabilising capacity of the hydrophobin protein in such a food product.

3.6. Mechanism of foam stabilisation using hydrophobin HFBII

Hydrophobin HFBII is known to be a highly surface-active protein: it reduces the a/w surface tension to about $30\ \text{mN m}^{-1}$ (Cox et al., 2007) and, once adsorbed, forms a highly elastic surface. This provides mechanical resistance to both coalescence and disproportionation. In general, the ability of any adsorbed protein or surfactant to produce

stable foams is related to both the structure of the molecule, the adsorption to the a/w surface, and the interactions between the molecules once adsorbed at the surface. However, there must be some underlying unique properties of HFBII that lead to such powerful foam-stabilising behaviour.

HFBII is known to have an approximately spherical structure, exhibiting both a hydrophilic part of the surface and, a smaller, hydrophobic part (Hakanpää, Linder, Popov, Schmidt, & Rouvinen, 2006). The internal structure of the protein consists of eight cysteine residues, which are arranged in such a way as to stabilise the structure and retain the globular shape of the protein, thus preventing significant unfolding (Linder et al., 2005). As a result, HFBII can be described as a small (ca. 2–3 nm diameter) molecule with a distinct amphiphilic nature, i.e. a protein Janus particle.

There has been increasing interest in the use of particles (including Janus particles) to stabilise emulsions and foams (Abkarian et al., 2007; Binks, 2002; Du et al., 2003; Fujii, Iddon, Ryan, & Armes, 2006; Hunter, Pugh, Franks, & Jameson, 2008; Murray, 2007; Murray & Ettelaie, 2004; Subramaniam, Mejean, Abkarian, & Stone, 2006), partly because such dispersions are potentially far more stable than those produced by surfactants. This is due to the energy of desorption being much greater for a particle than for a smaller surfactant molecule. Although the general model of particle stabilisation may go some way in describing the behaviour of hydrophobin as a foam stabiliser, there is still much to be understood in terms of the adsorption behaviour of this protein to the a/w surface, the film elasticity, and the exceptional stabilising capability that ensues. For example, the nature of interactions between hydrophobin molecules once adsorbed at the a/w surface is currently not well understood. Such interactions may also play a significant role in the mechanical properties and, therefore, bubble and foam stabilisation.

4. Conclusions

We have demonstrated the exceptional stabilisation of aqueous foams using hydrophobin HFBII, at a concentration of only 0.1 wt% protein and across a range of solution pH conditions. In the presence of a thickening agent to slow the rate of creaming, hydrophobin can stabilise foam to the extent where little, or no, air phase loss is observed, for over 4 months. In fact, we have an example where most of the air phase volume remains after 2.5 years storage at chill temperature. The stability of such foams is well in excess of those stabilised using other common food-aerating/emulsifying agents, such as milk proteins and Tween. We have also shown the potential of hydrophobin as a foam stabiliser in one example food product, a chocolate milk shake.

The mechanism of foam stabilisation using HFBII is believed to originate through surface adsorption of this protein and the resulting high surface elasticity that results,

i.e. there is no affect of this protein on the bulk phase rheology. Although more work is required to understand the surface behaviour, we propose that HFBII may act as a nature-designed Janus-like particle, adsorbing strongly to the a/w surface, and effectively resisting surface changes due to processes such as disproportionation. This is in contrast to milk proteins, which exhibit a relatively low elasticity and have relatively little effect on slowing the rate of disproportionation. A more detailed model of the physical properties of hydrophobin at surfaces and interfaces is required, however, in order to fully understand its mechanistic behaviour and further exploit the potential of this class of proteins in a variety of technical applications.

The ability to create foams that exhibit exceptional bubble stability at low concentrations over a period of several weeks or even months could lead to a number of new applications. In foods, the ability to produce foam of greater stability may lead to improved storage stability as well as improvements in physical and sensory properties of products such as ice cream, sorbets, and low-fat whipping cream. Moreover, use of hydrophobins could further lead to the possibility of aerating food products that currently do not contain air because of instability problems: for example, mayonnaise, shelf-stable milk shakes, smoothies and other beverages, yoghurt, and gelatine-free mousse. As a result, benefits such as fat/calorie reduction or improved/new product textures are possible.

Finally, hydrophobins are a class of fungal proteins some of which are presently consumed: for example, hydrophobins are present in mushrooms. Therefore, there is significant potential for hydrophobins, in an isolated form, to be used in food products.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.foodhyd.2008.03.001](https://doi.org/10.1016/j.foodhyd.2008.03.001).

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