# Control of Surfactant-Induced Destabilization of Foams through Polyphenol-Mediated Protein–Protein Interactions

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The effectiveness of (+)-catechin in the control of foam stability of a deliberately destabilized Tween 20/β-lactoglobulin mixed system was investigated. Significant improvement in the foamability and foam stability was obtained in the presence of low concentrations of (+)-catechin. The maximum improvement was observed at a molar ratio of (+)-catechin to protein of approximately 0.1. The mechanism of action of (+)-catechin was investigated by careful study of the properties of thin liquid films (isolated foam lamellae). This polyphenolic compound was found to increase the equilibrium thickness of the films, slow the rate of drainage, and slow the diffusion of a fluorescent probe which was located in the adsorbed layer. The data are consistent with (+)-catechin-induced cross-linking of proteins in the adsorbed layer. Such molecules offer considerable potential for the control of protein foam stability.

**Keywords:** *Restabilization; immobile; interactions; foam stability; interface*

## INTRODUCTION

Food emulsions and foams often consist of a complex mixture of proteins and low molecular weight surfactants. Both classes of molecules demonstrate completely distinct mechanisms of interfacial stabilization (Ewers and Sutherland, 1952; Halling, 1981). Proteins stabilize dispersed systems such as foams or emulsions by formation of a "tough" viscoelastic film at the interface. Examples of food foams where this can occur include beer, bread, and meringues. Mechanical stability is conferred by this adsorbed layer, and this prevents bubble rupture. Instability occurs if there is competitive adsorption of low molecular weight components at the interface, which disrupts the protein–protein interactions that are the basis of the viscoelastic properties (Courthaudon *et al.*, 1991). Thus, destabilization of the interface and dispersion occur when surface active lipids (Daftary *et al.*, 1968; MacRitchie and Gras, 1973) or surfactants (Clark *et al.*, 1992; Tornberg and Lundh, 1981) compete to occupy space in the adsorbed layer with proteins in the same system.

The Tween 20/β-lactoglobulin (β-Lg) system (Wilde and Clark, 1993) is an example of a model competitive adsorption system. Tween 20 is the name given to the polysorbate emulsifier polyoxyethylene (20) sorbitan monolaurate, while β-Lg is the major protein found in the whey fraction of milk. The surface activity of the Tween 20 can lead to preferential adsorption of this molecule at the interface. Inclusion of comparatively small quantities of Tween 20 in the protein solution disrupts protein–protein interactions at the interface, leading to the onset of lateral diffusion of the protein in the adsorbed layer and the subsequent destabilization of foams and foam films. At a molar ratio of Tween 20: β-Lg ($R$) of 0.25, the stability of the foams is measurably reduced while the lateral diffusion of proteins at the interface is initiated, indicating partial disruption of the viscoelastic interfacial multilayers. This model system is a simplification of the stabilizing protein layer in "real" systems contaminated or brought into contact with lipids and/or surfactants.

Tannin is a term used to collectively describe and group polyhydroxyphenolic compounds of approximate molecular mass 500–3000 Da found in plant material (Dreher, 1987; Porter, 1989). Tannins can be divided into two groups (i) "hydrolyzable", which yield a polyhydric alcohol and gallic acid or related compounds and (ii) "condensed", comprising flavonoid compounds, one of which is the precursor (+)-catechin. Self-initiated condensation polymerization of (+)-catechin ($C_{15}H_{14}O_6$) produces red-brown tannins, the intensity of which is concentration dependent. Catechins themselves belong to a group of compounds referred to as flavan-3-ols, which exist as a number of isomers depending on the positioning of functional groups. (+)-Catechin is routinely found in a number of plant sources such as stems, bark, the aleurone layer of seeds and grains, and lignified or damaged tissues. Tannins, including catechins, are thought to have antifungal/pathogenic properties within plant tissue (Swain, 1965) and feature particularly in ripening and dying/dead cells and material. The astringency associated with some fruit, cocoa, and tea is thought to be attributable to tannins, which complex with glycoproteins in saliva and polypeptides within the mouth. Fresh tea infusion may contain 25–35% tannins on a dry weight basis of which (+)-catechin may account for 1–2% (Sanderson, 1972). Dietary proteins have been shown to complex with tannins, resulting in protein precipitation in beers, wines, and fruit juices (Wong, 1973). Studies with complex mixtures of tannins show that tannin components bind to proteins and can induce cross-linking (Ricardo-da-Silva *et al.*, 1991; Sekiya *et al.*, 1984).

The aim of this study is to investigate the potential of polyphenol- and specifically (+)-catechin-mediated protein–protein interactions in enhancing the robustness of the adsorbed protein layer in a model system, comprised of a mixture of Tween 20 and β-Lg. The ratio of these two components was carefully selected to ensure that the foaming properties of the system were deliberately compromised to allow clear indication of the

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effect of the presence of (+)-catechin on foaming properties and to allow elucidation of the mechanism of action of the polyphenol.

## MATERIALS AND METHODS

$\beta$-Lg (L-0130; 3× crystallized and lyophilized) and (+)-catechin (C-1251) were purchased from Sigma Chemical Co. Tween 20 [No. 28320 Surfact-Amps 20; 10% solution polyoxyethylene (20) sorbitan monolaurate] was purchased from Pierce, and 5-*N*-(octadecanoyl)aminofluorescein (No. O-322) was obtained from Molecular Probes Inc. All samples were prepared using 10 mM sodium phosphate buffer using surface chemically pure distilled water adjusted to pH 7.0. We refer to the composition of solutions by an $R$ value, which is the molar concentration ratio of Tween 20 to $\beta$-Lg. In the majority of experiments, the protein concentration was held constant at 1 mg/mL (54.3 $\mu$M). The Tween 20 concentration was also held constant at 13.58 $\mu$M.

**Foaming.** Foam stability was assessed using a microconductivity method (Clark *et al.*, 1991a). Two milliliters of protein solution was sparged with pure nitrogen until the generated foam reached a predetermined mark, 2.8 cm above the body of the liquid. Foam microconductivity remaining 5 min after sparging had ceased ($C_{300}$) was used as a measure of foam stability (Coke *et al.*, 1990), while the microconductivity immediately after sparging had ceased ($C_0$) was used as a estimate of the ability of the solution to generate a foam (foamability).

**Preparation of Thin Films for Drainage Behavior and Measurment of Equilibrium Thickness.** Thin films (isolated foam lamellae) were formed in a small ground glass annulus contained in a temperature- and humidity-controlled housing as previously described (Clark *et al.*, 1990). After a droplet of the solution of interest had equilibrated for 30 min, the thin film was created by withdrawal of some of the liquid from the droplet in the ring. Drainage behavior was examined under epi-illumination using an inverted microscope. An interferometric method described earlier (Clark *et al.*, 1989, 1990) was used to determine film thickness.

**Fluorescence Recovery after Photobleaching (FRAP).** The FRAP technique was used to investigate the lateral diffusion behavior of adsorbed layers in air–water thin films. The fluorescent surface active probe molecule 5-*N*-(octadecanoyl)aminofluorescein (ODAF), which preferentially accumulates at the interface, was used as a probe of interfacial structure (Coke *et al.*, 1990). The low concentrations of ODAF used in these experiments do not interfere with the adsorption of other species. The mobility of this "reporter" molecule in the interfacial layer can be used as a qualitative probe of the extent of protein–protein interaction in the adsorbed layer. In these experiments, the $R$ value of the solution was increased to 0.5 to maximize breakdown of protein–protein interactions. Thus, the $\beta$-Lg concentration was maintained at 1 mg/mL but the Tween 20 concentration was increased to 27.15 $\mu$M.

**Surface Dilational Measurements.** The surface dilation properties were measured according to the method of Kokelaar *et al.* (1991). The apparatus involves a periodic interfacial expansion and compression resulting from raising and lowering a 10 cm diameter ground glass ring into a vessel containing 20 mL of the test solution. The large volumes of solution required for these measurements necessitated a reduction in solute concentration. Measurements were made using constant protein concentrations of 0.02 (1.09 $\mu$M) and 0.1 mg/mL (5.43 $\mu$M) with Tween 20 concentrations of 0.272 and 2.57 $\mu$M, respectively.

## RESULTS

**Foaming Properties.** Foams formed from the Tween 20/$\beta$-Lg solution ($R$ = 0.25) showed increased foamability in the presence of added (+)-catechin up to a concentration of 7 $\mu$M (Figure 1). The initial foam conductivity ($C_0$) was greater as a result of increased foam density, but the time to create the required volume of foam was greater in solutions containing (+)-catechin (results not shown). Notably, solutions containing (+)-catechin alone under the same concentrations as those examined with the mixed system did not produce a measurable foam. Foamability did decline in solutions containing (+)-catechin at concentrations above 7 $\mu$M. Nevertheless, the equilibrium value reached was still somewhat greater than observed in the solution containing no (+)-catechin (i.e. Tween 20/$\beta$-Lg alone).

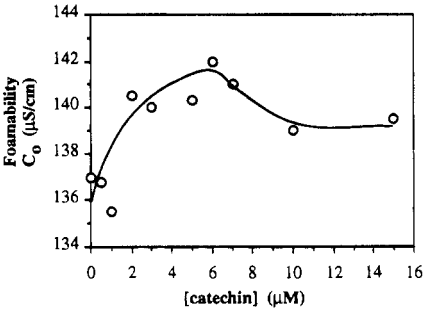


**Figure 1.** Foamability as determined by initial foam conductivity ($C_0$) of solutions of Tween 20/$\beta$-Lg ($R$ = 0.25) as a function of the concentration of (+)-catechin.The symbols represent data points. The solid curve is included as an aid to the eye and not a fit to the data points.

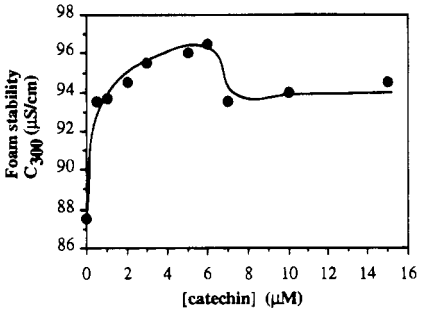


**Figure 2.** Foam stability determined by residual conductivity of the foam after 300 s of drainage of samples of Tween 20/$\beta$-Lg ($R$ = 0.25) as a function of (+)-catechin concentration. The symbols represent data points. The solid curve is included as an aid to the eye and not a fit to the data points.

The presence of (+)-catechin in the Tween 20/$\beta$-Lg test solution ($R$ = 0.25) increased foam stability at very low concentrations of 0.5 $\mu$M (molar equivalent to protein, $R_{cat}$ = 0.01) as shown in Figure 2. Higher concentrations of (+)-catechin increased the foam stability to a pseudoplateau with maximum stability observed at 5–6 $\mu$M. The stability of the foams formed from solutions containing 6 $\mu$M (+)-catechin [(+)-catechin:$\beta$-Lg ratio, $R_{cat}$ = 0.11] was enhanced by approximately 11% compared with that characteristic of the Tween 20/$\beta$-Lg system alone. At marginally higher concentrations than 6 $\mu$M added (+)-catechin, there was a reduction in the enhancement of foam stability to levels comparable with those observed following addition of 0.5 $\mu$M (+)-catechin alone. Nevertheless, the enhancement was still significant in comparison with the foam stability of solutions of Tween 20/$\beta$-Lg alone.

**Thin Film Drainage.** Thin films formed from solutions of Tween 20/$\beta$-Lg alone ($R$ = 0.25) generally showed transitional drainage behavior consistent with the Tween-induced breakdown of protein–protein interactions in the interfacial layer (Clark et *al.*, 1991a; Coke et *al.*, 1990). Typically the films contained rigid regions, rich in protein–protein interactions in an otherwise mobile fluid-like film which was depleted in protein–protein interactions. The rigid regions were

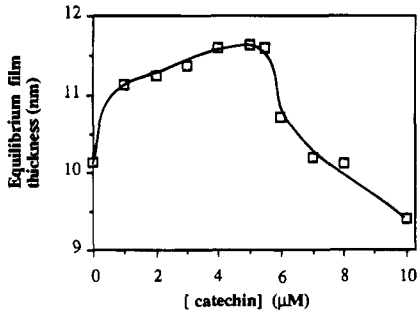


**Figure 3.** Influence of (+)-catechin concentration on equilibrium film thickness of foam lamellae formed (thin films) from solutions comprised of mixtures of Tween 20/β-Lg ($R$ = 0.25). The symbols represent data points. The solid curve is included as an aid to the eye and not a fit to the data points.

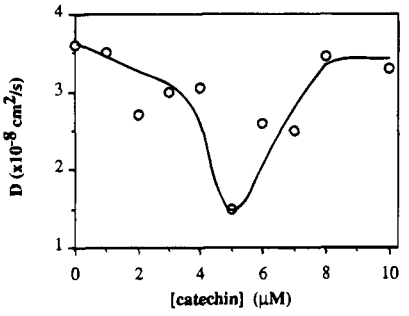


**Figure 4.** Effect of (+)-catechin concentration on the lateral diffusion coefficient ($D$) of the fluorescent probe ODAF in the adsorbed layer of thin films stabilized by solutions of Tween 20/β-Lg ($R$ = 0.5). The symbols represent data points. The solid curve is included as an aid to the eye and not a fit to the data points.

progressively ejected from the film. Occasionally films exhibited immobile behavior. The presence of (+)-catechin at concentrations <3 μM did not alter the nature of the thin film drainage. However, at 4 μM and even more noticably at 5 μM (+)-catechin, the thin film drainage behavior altered significantly, consistent with rigidification of the adsorbed layers.

**Film Thickness.** The characteristic thickness of equilibrium films formed from Tween 20/β-Lg ($R$ = 0.25) solutions was approximately 10 nm. Addition of 1 μM (+)-catechin (Figure 3) increased film thickness by about 10%. The characteristic film thickness steadily increased to a maximum of over 11.5 nm at (+)-catechin concentrations between 4 and 5.5 μM. Further addition of (+)-catechin resulted in a gradual reduction in equilibrium film thickness to approximately that of the system in the absence of any (+)-catechin. At 10 μM (+)-catechin, the equilibrium film thickness was found to be less than that of films formed from Tween 20/β-Lg alone.

**Lateral Diffusion in the Adsorbed Layer.** A reduction in the lateral diffusion of ODAF was observed in the adsorbed layers of thin films formed from the Tween 20/β-Lg solution with increasing concentration of (+)-catechin (Figure 4). The lateral diffusion coefficient ($D$) was only gradually reduced to 3 × $10^{-8}$ $cm^2/s$ with increasing concentrations of (+)-catechin up to and including 4 μM. However, at 5 μM (+)-catechin a large reduction in $D$ to 1.5 × $10^{-8}$ $cm^2/s$ was observed which coincides with the maximum in equilibrium film thickness. Further increases in concentrations of added (+)-catechin reversed this trend and caused an increase in $D$ of ODAF within the adsorbed layers. Nevertheless, between 6 and 6.5 μM (+)-catechin lateral diffusion of ODAF was still lower than that observed at 4 μM (+)-catechin and still significantly lower than that observed in the $R$ = 0.5, Tween 20/β-Lg system alone. The lateral diffusion coefficient of ODAF remained lower in thin films of solutions containing 8−10 μM (+)-catechin than in the thin films containing no (+)-catechin but was of similar magnitude to that characteristic of the system containing 1−4 μM (+)-catechin. This is consistent with the foam stability data, where high levels of (+)-catechin produce a small increase in foam stability similar to that observed with low concentrations of (+)-catechin.

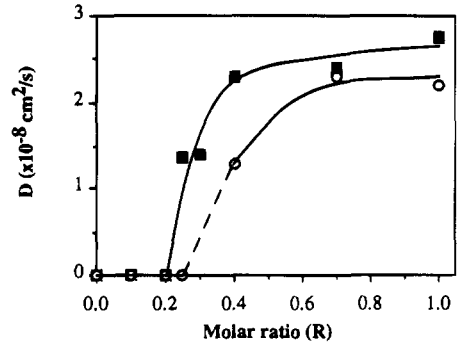


**Figure 5.** Lateral diffusion coefficient ($D$) of ODAF in the adsorbed layer of thin films formed from solutions of β-Lg (54.3 μM) in the absence (■) and presence of (+)-catechin (5 μM) (○) as a function of increasing molar ratio ($R$) of Tween 20. The symbols represent data points. The solid curves are included as an aid to the eye and not a fit to the data points.

In a separate series of FRAP experiments the concentration of Tween 20 was varied in solutions containing fixed concentrations of protein (54.3 μM), in the absence and presence of a constant (+)-catechin concentration (5 μM). The results are presented in Figure 5. The lateral diffusion coefficient of ODAF was found to be dependent on the presence or absence of (+)-catechin at certain $R$ values. In the system containing no (+)-catechin, diffusion started at $R$ = 0.25 and rose sharply to $R$ = 0.4, where it attained an equilibrium value which only increased gradually with further additions of Tween 20.

The presence of (+)-catechin delayed the onset of mobility in the Tween 20/β-Lg thin films. In the system containing (+)-catechin, ODAF was immobile in thin films of solution composition $R$ = 0.25 and was either mobile or immobile at $R$ = 0.3 depending on whether the drainage properties of the films were fluid (surfactant-like) or rigid (protein-like) in behavior. At solution compositions of $R \geq 0.4$, diffusion of ODAF was always observed and the films always possessed mobile, surfactant-like drainage properties. The magnitude of $D$ increased with Tween concentration but reached a maximum value at approximately $R$ = 0.7. It is notable that $D$ of ODAF in samples containing (+)-catechin was always significantly lower than in the absence of (+)-catechin. Indeed, the maximum $D$ value observed was about 20% lower at 2.2 × $10^{-8}$ $cm^2/s$ in the system containing (+)-catechin at $R$ = 1.0.

**Surface Dilational Properties.** Evidence for a detectable increase in surface elasticity or viscosity in solutions containing 0.5 μM (+)-catechin is presented in Figure 6. In the $R$ = 0.25 system containing 0.02 mg/mL protein and no (+)-catechin, the elastic modulus was only about 55% of that obtained for the same system but with 0.05 μM (+)-catechin included. Higher concentrations of (+)-catechin also increased surface elasticity compared to the control, but the maximum effect was observed at 0.05 μM (+)-catechin. Surface dilational viscosity values also showed the greatest

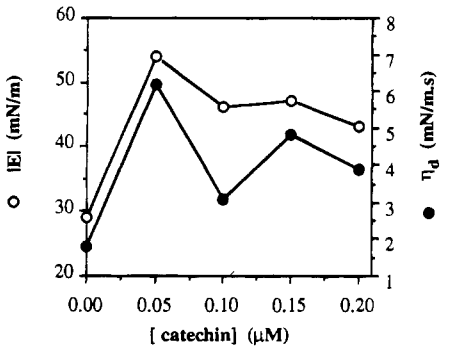


**Figure 6.** Changes in the dilational properties of the air–water interface formed on solutions of Tween 20 (0.272 $\mu$M) and $\beta$-Lg (1.086 $\mu$M) ($R$ = 0.25) as a function of (+)-catechin concentration. The elastic modulus ($|E|$) and the surface dilational viscosity ($\eta_d$) are represented by the symbols (○) and (●), respectively.

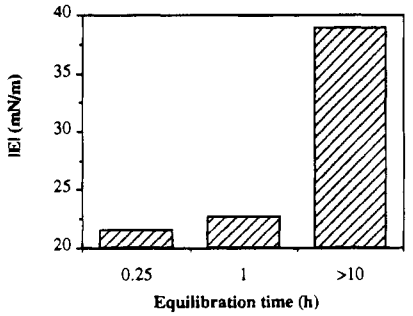


**Figure 7.** Time-dependent changes in the elastic modulus ($|E|$) of the air–water interface of solutions of Tween 20 (2.58 $\mu$M) and $\beta$-Lg (5.43 $\mu$M) ($R$ = 0.475) in the presence of 5 $\mu$M (+)-catechin.

increase in the presence of submicromolar levels of (+)-catechin. However, absolute values of the suface dilational viscosity show poor reproducibility compared with the elastic modulus. The time dependence of the change in surface dilational properties was studied in separate experiments conducted at higher Tween 20 concentrations ($R$ = 0.47). The results are shown in Figure 7. The-time dependent increase in surface elastic modulus resulting from the presence of 5 $\mu$M (+)-catechin is very clear. Similar effects were observed during FRAP measurements.

## DISCUSSION

It is now firmly established that competitive adsorption of low molecular weight surfactants results in the breakdown of protein–protein interactions in the adsorbed layers of foam films (Clark *et al.*, 1989, 1991a; Coke *et al.*, 1990). The subsequent reduction in the viscoelastic properties of the interfacial layer negates the primary mechanism for the stabilization of protein foams with consequent reduction in foam stability. In addition, the protein aggregates remaining in the interfacial layer as "islands" impede surfactant mobility in the adsorbed layer with a consequent reduction in film stabilization by the Marangoni mechanism. There are two possible strategies that may be employed to control surfactant-induced destabilization of foams. First, a component, preferably of natural origin, could be added that selectively binds the low molecular weight species, preventing its adsorption at the interface. We have reported the successful application of a lipid binding protein, puroindoline, from wheat in such a role, in the stabilization of beer foam (Clark *et al.*, 1994) and egg white protein foams (Husband *et al.*, 1994). An alternative strategy is to enhance protein–protein interactions in the adsorbed layer in a manner that increases the resistance to disruption of protein–protein interactions in the adsorbed layer by competitive adsorption of surfactants. This latter approach has been shown to be effective in the stabilization of model beer systems through hop iso-α-acid-mediated cross-linking of adsorbed proteins (Clark *et al.*, 1991b). This paper extends this work by examination of the potency of another class of natural plant-derived molecules capable of cross-linking proteins, namely the polyphenols.

Partially destabilized foams formed from solutions containing Tween 20 and $\beta$-Lg provide an ideal test system for the screening of potential cross-linking molecules. In this study we have found a good correlation between the observed increase in foamability and stability and changes in the thin film properties. The rise in foamability reflects an increase in foam density due to increased bubble stability. In addition, the increase in the $C_0$ value with (+)-catechin in the concentration range 1–6 $\mu$M may also reflect the increase in film thickness through this concentration range. The observed increase in equilibrium film thickness infers an increase in the adsorbed layer thickness at the interface resulting from inclusion of (+)-catechin. Such an effect will also slow the rate of film drainage, particularly if there is an increase in the rigidity of the interfacial layers due to cross-linking. The strongest evidence for (+)-catechin-mediated cross-linking is the decrease in the diffusion coefficient of ODAF in the adsorbed layers of the thin film. Our hypothesis is that this effect is brought about by the restriction of diffusion of the probe by the formation of (+)-catechin-mediated protein–protein cross-links in the adsorbed layer. Comparatively low levels of cross-linking, as determined by the small reduction in the diffusion coefficient in the presence of 1–2 $\mu$M catechin are sufficient to cause a major improvement in foam stability (Figure 2).

The precise chemical nature of the cross-link between (+)-catechin and $\beta$-Lg is unclear. (+)-Catechin is known to self-associate (Porter, 1989) and to interact with proteins through hydrogen bond formation. It is possible that hydrophobic, covalent, and electrostatic interactions (Ricardo-da-Silva *et al.*, 1991; Sekiya *et al.*, 1984) may also have a role to play, as has been reported for tannins and their precursors (Clark *et al.*, 1991b; Gramshaw, 1969). Recent studies (McManus *et al.*, 1985) and unpublished measurements of our own involving photon correlation spectroscopy studies in the presence of chaotropic agents indicate both hydrogen bonding and hydrophobic interaction are implicated in the functionality of (+)-catechin. This assertion is supported by the absence of detectable higher molecular weight bands in samples in the presence of (+)-catechin in polyacrylamide electrophoresis gels run under denaturing conditions. Interactions between (+)-catechin and $\beta$-Lg may serve to increase the amount of water associated with the adsorbed layer, again thickening the adsorbed layer and resultant foam films with the consequent increase in foam stability (Velev *et al.*, 1993).

A maximum in foamability, foam stability, and film thickness and a minimum in surface diffusion of ODAF are all observed at approximately 5 $\mu$M (+)-catechin. A definitive explanation for this effect has not been found. However, our favored hypothesis is the following. We assume that the protein must possess a number of binding sites for catechin and that the cross-linking

activity of this molecule derives from its multivalency. Crosslinking of proteins in the interfacial layer relies upon the interaction of protein-bound catechin molecules with vacant interaction sites on neighboring protein molecules. At a certain (+)-catechin concentration the interaction sites on the neighboring protein molecules will be saturated with (+)-catechin and impede the formation of (+)-catechin bridges between the protein molecules. Further work is required to explain why the effect is observed at comparatively low (+)-catechin concentrations (i.e. where the molar concentration of catechin constitutes only 10% of that of the protein). It may be that the presence of both free protein and Tween 20/protein complex plays a role in this effect.

Consideration of the extent of complex formation is necessary when the data from the dilational study are evaulated. Previous work has shown that adsorbed Tween 20/$\beta$-Lg complex is responsible for the breakdown of protein−protein interactions in the adsorbed layers of this system with a subsequent reduction in foam stability (Clark *et al.*, 1993; Coke *et al.*, 1990). The latter experiments were all performed at a protein concentration of 10.9 $\mu$M and the molar ratio at which protein lateral diffusion was first observed in the adsorbed layer was $R = 0.9$. At higher protein concentrations such as the 1.0 mg/mL solutions used in the thin film experiments here, disruption of the adsorbed layer occurs at lower Tween 20 concentrations, due to the increased levels of complex formed (Clark *et al.*, 1992). However, this effect caused some complications when we started with a lower total protein concentration as in the dilation experiments, as it is impossible to construct a solution that has identical composition in terms of the relative concentrations of free protein, Tween 20, and complex to that present in the 1.0 mg/mL solutions used in the thin film experiments. Under the solution conditions chosen for the surface dilational measurements (0.02 mg/mL $\beta$-Lg and 0.272 $\mu$M Tween 20), there is approximately 0.05 $\mu$M complex present in solution (i.e. 4.6% of the total protein) compared to 22.5% complexed protein under conditions used in the thin film experiments. Thus, it is not surprising that the relative concentration of (+)-catechin required to increase surface elasticity and viscosity is much lower on a mole to mole basis than required to produce optimal effects in the thin film studies.

We have established the principle that a polyphenolic compound can enhance foam stability by altering interfacial layer structure through interactions with adsorbed proteins. It should be stressed that our studies have been confined to a model system and additional investigations should be undertaken to identify potential applications of these findings in real food systems. Since polyphenolic compounds are found naturally in plant material, potential applications of this work should focus on foods and beverages produced from or containing processed plant material. The latter will be necessary to release polyphenolic compounds from their interactions with polysaccharides in plant cell walls. There are some beverages that meet these criteria. For example, polyphenolic compounds are released during the brewing of beer. Traditionally, they have been considered in a rather negative way as they have been associated with haze production caused by polyphenol-induced protein precipitation. Our findings suggest that they may also behave in a positive manner in stabilizing beer foam, which according to brewers is an important factor in the consumer perception of quality in this product. Possible food applications could include baked products produced using wholemeal flour which contains phenolic compounds present in the aleurone layers of grain. There may be areas of application of this concept in the development of new foamed foods and beverages.

## CONCLUSIONS

(1) The presence of (+)-catechin in solutions containing mixtures of Tween 20 and $\beta$-Lg significantly enhances the foamability and foam stability of these samples.

(2) Inclusion of (+)-catechin results in an increase in the thickness of foam lamellae (thin films) and reduces the rate of drainage.

(3) Evidence from surface diffusion measurements supports the formation of (+)-catechin-mediated protein−protein interactions in the interfacial layer.

(4) The long-range effect of cross-link formation was confirmed by the increase in the surface dilational elasticity and viscosity.

(5) This work provides preliminary evidence to suggest that polyphenolic compounds may have potential as functionality enhancing molecules.

## ACKNOWLEDGMENT

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