

Enhancement of Protein Foam Stability by Formation of Wheat Arabinoxylan-Protein Crosslinks

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ABSTRACT

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The foam stability properties of a defined mixed solution of Tween 20 and bovine serum albumin was evaluated as a function of arabinoxylan concentration. A marked increase in the foam stability was observed with low concentrations of arabinoxylan. Maximum improvement in the foam stability was obtained with 0.2–0.3 mg/mL of arabinoxylan. Enhancement of foam stability due to a combination of bulk viscosity changes and surface effects was identified. The relative contribution of arabinoxylan to bulk viscosity and adsorbed layer structure was studied by examination of the properties of thin liquid films and the macroscopic air-water interface.

Arabinoxylan reduced the rate of thin film drainage, increased the equilibrium thickness of the films, slowed the lateral diffusion of a fluorescent probe molecule located in the adsorbed layer, and increased the surface elasticity. These data are congruent with arabinoxylan-mediated crosslinking of adsorbed protein. These observations may be of significance in gas retention during breadmaking. In addition, this naturally occurring polysaccharide offers potential for use in the control of protein foam stability.

Individually, both proteins and surfactants form stable foams. However, in general, when they are mixed rather than behaving in an additive manner (producing a more stable foam due to the increase in total surfactant present), they frequently produce a foam of much more transient stability. The reason for this has emerged over recent years; it derives from the inherent incompatibility of the two separate mechanisms that serve to stabilize the foam films formed by two surface active agents.

At relatively low concentrations, the low molecular weight surfactant competes with the protein to form the adsorbed layer. This competitive adsorption of low molecular weight amphiphiles, such as food emulsifiers or surfactants, is now established as a significant factor in the disruption of protein-protein interaction at the interface (Courthaudon et al 1991, Chen et al 1993), reduction in surface rigidity (Clark et al 1993), and consequent reduction in protein foam stability (Clark et al 1991).

Bovine serum albumin (BSA) is a globular protein with a monomer molecular mass of 66.2 kDa (Peters 1980). The protein possesses significant foaming and surface active properties (Fox 1989, Kinsella and Whitehead 1989) and has the ability to bind a broad range of ligands such as surfactants, lipids, nucleotides, and ionic species (Peters 1980). At pH values >4.9–5.1, which is the isoelectric point of the protein, the molecule possesses a net negative charge.

The formation of polysaccharide-protein complexes is now a well-established phenomenon for some polysaccharide-protein mixtures (Dickinson and Galazka 1991; Dickinson 1993, 1994). A comprehensive review of common polysaccharide-protein mixtures used in food dispersions has been published (Stainsby 1980). Polysaccharides are often responsible for increases in the bulk viscosity of food and this can slow down liquid drainage from a foam.

The fibrous and structural material that constitutes plant cell walls is composed of three main groups of polymers: cellulose, β -glucans, and hemicelluloses (pentosans). Arabinoxylan is a component of the pentosan fraction and is composed of mainly pentose sugars. Water-soluble and water-insoluble varieties of arabinoxylan have been extracted from cereal grains. Wheat arabinoxylans are linear polymers composed of D-arabinose, D-xylose, and small quantities of esterified ferulic acid (Eliasson and Larsson 1993, Rybka et al 1993).

Despite the fact that $\approx 80\%$ of the ferulic acid is located in the bran fraction (0.7 mg/g of whole grain) of the wheat grain (Izydorkzyk and Biliaderis 1993, Rybka et al 1993), concentrations of ≈ 0.1 mg of ferulic acid/g of whole grain have been recorded in wheat flour (Yeh et al 1980, Rybka et al 1993). Soluble wheat arabinoxylan has been positively linked with enhancement of loaf volume and the baking properties of bread (Hoseney et al 1969). The mode of action of the soluble arabinoxylan fraction in increasing dough protein functionality is due to two properties of the pentosan: 1) the water-binding capacity alters the bulk rheological properties of the matrix separating foam bubbles; 2) an oxidative gelation mechanism (Markwalder and Neukom 1976; Gruppen et al 1993a,b) between aromatic residues on the protein and the ferulic acid (Izydorkzyk et al 1990, Moore et al 1990) of the arabinoxylan has been proposed, and this may explain the decrease in loaf volume when arabinoxylan was removed. Mixing of dough (Yeh et al 1980) with oxidizing agents such as hydrogen peroxide (Hoseney and Faubion 1981, Izydorkzyk et al 1990) was reported to cause oxidative gelation of the arabinoxylan.

In this article, we examine the effect of a soluble arabinoxylan fraction on the foam stability of solutions containing a mixture of protein, BSA, and the surfactant Tween 20. In addition, we report on studies concerning the influence of arabinoxylan on the structure of the adsorbed layer in this model multicomponent system to allow delineation of surface and bulk effects.

MATERIALS AND METHODS

Purified protein bovine serum albumin (BSA, A-7030, >98% purity, 0.006% fatty acid) was purchased from Sigma Chemical Co. (Poole, Dorset, UK). Tween 20 (No. 28320 Surfact-Amps 20, 10% solution polyoxyethylene [20] sorbitan monolaurate) was purchased from Pierce (Rockford, IL), and 4-(N,N-dioctyl)amino-7-nitrobenz-2-oxa-1,3-diazole (DiO-NBD, No. D-70) was obtained from Molecular Probes Inc. (Eugene, OR). Purified wheat flour arabinoxylan (Axn, pentosan, type MWP90801) was purchased from the MegaZyme Pty. Ltd. (Warriewood, NSW, Australia). All samples were prepared using 10 mM citric acid-sodium phosphate buffer using surface chemically pure distilled water adjusted to pH 5.6. We refer to the composition of solutions by an *R* value, which is the molar concentration ratio of Tween 20 to BSA. In the majority of experiments, the protein concentration was held constant at 4.08 mg/mL (61.8 μ M). The Tween 20 concentration was also held constant at 25 μ M.

In this article, we report a detailed study of the effect of arabinoxylan concentration on the stability of deliberately unstable foaming system formed from a defined mixture of the low molecular weight

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surfactant Tween 20 and BSA. Separate studies of the properties of isolated foam films and the surface dilational behavior of these systems has allowed delineation of surface and bulk effects.

Foaming

Foaming properties were assessed using a microconductivity method (Clark et al 1991, Wilde 1996). Protein solution (2 mL) was sparged with pure nitrogen until the generated foam reached a pre-determined mark 2.8 cm above the body of the liquid. The foam conductivity remaining 5 min after sparging had ceased (C_{300}) was used as a measure of foam stability (Coke et al 1990), while the microconductivity recorded immediately after sparging had ceased (C_0) was used as an estimate of the ability of the solution to generate a foam (foamability). Where the bulk conductivity of solutions used to form foams varied with solution composition, the foam stability was normalized as: $(\% \Delta C) = 100(C_{300}/C_0)$.

Preparation of Thin Films for Drainage Studies and the Measurement 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 formed by withdrawal of some of the liquid from the droplet in the ring. Drainage behavior was examined under epi-illumination with an inverted microscope. An interferometric method described earlier (Clark et al 1989,1990) was used to determine film thickness.

Fluorescence Recovery After Photobleaching

The fluorescence recovery after photobleaching (FRAP) technique was used to investigate the lateral diffusion of molecules in the adsorbed layers in air-water thin films. The fluorescent surface active probe molecule DiO-NBD, which preferentially accumulates at the interface, was used as a probe of interfacial structure (Coke et al 1990). The low concentrations of DiO-NBD 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 0.46 to maximize breakdown of protein-protein interactions. Thus the BSA concentration was maintained at 8.16 mg/mL, while the Tween 20 concentration was increased to 56.9 μ 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 250 mL of the test solution. The large volumes of solution required for these measurements necessitated a reduction in solute concentration. The capability of polysaccharide to crosslink protein in the adsorbed layer was examined. A protein solution (1 mg/mL) was allowed to equilibrate in a humidity- and temperature-controlled environment at a constant temperature of 18°C. After attainment of a constant surface tension and dilational modulus, an aliquot of the potential crosslinking agent solution was introduced in the sub-phase and mixed uniformly into the protein solution. The mixing procedure was performed without disruption of the surface by thoroughly dispersing the crosslinking agent solution in the protein solution outside the ground glass ring. The dilational properties of the interface were then monitored as a function of time.

Kinematic Viscosity

The relative viscosity of a liquid was determined using a kinematic viscometer (SLSU, type 3, 0.744 mm diameter capillary

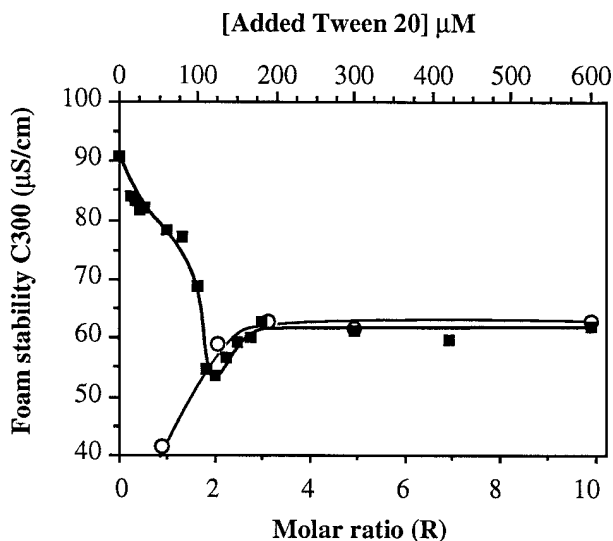


Fig. 1. Foam stability (C_{300}) of solutions of bovine serum albumin (BSA) (4.08 mg/mL, 61.8 μ M) as a function of Tween 20 concentration (\blacksquare), expressed as molar ratio R (Tween 20 to BSA). Data for Tween 20 alone (\circ) are also shown. All samples prepared in 10 mM citric acid and sodium phosphate buffer pH 5.6.

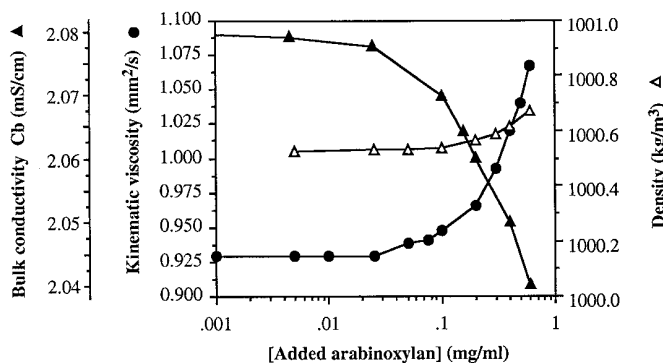


Fig. 2. Kinematic viscosity (\bullet) and density (Δ) of solutions of arabinoxylan as a function of concentration. Also shown is the effect on bulk conductivity (\blacktriangle) of Tween 20 and bovine serum albumin (BSA) (molar ratio $R=0.42$, foam test solution at pH 5.6).

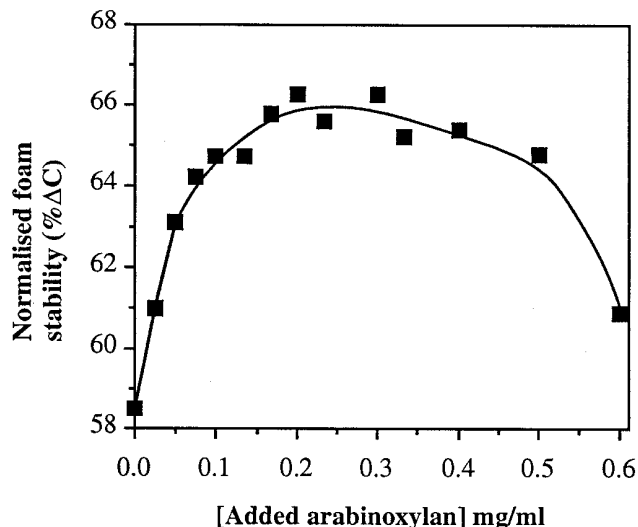


Fig. 3. Normalized foam stability data for foams formed from solutions containing 4.08 mg/mL of bovine serum albumin (BSA) and 25 μ M Tween 20 (molar ratio $R=0.42$) as a function of added arabinoxylan.

from PSL Scientific Laboratories). The time for the liquid to flow through a calibrated capillary is related to the kinematic viscosity (Shaw 1992). Solutions were accurately thermostated and allowed to equilibrate for 30 min at 25°C before measurement.

Solution Densitometry

The density of solutions of interest was measured using a PAAR Scientific density meter (DMA60) and density measuring cell (DMA602) equipped with precision thermometer (ASL F25) and the temperature was controlled at $20 \pm 0.1^\circ\text{C}$. A spreadsheet package on a Viglen personal computer was used to calculate the density of the test solution.

Surface Tension Measurements

The surface tension of dilute solutions of wheat arabinoxylan was measured using a thermostatically controlled Krüss Digital Tensiometer (model K10T) equipped with a du Nuüy ring at 20°C.

RESULTS

Foaming, Kinematic Viscosity, and Density Measurements

Foam stability (C_{300}) data for BSA solutions ($61.8 \mu\text{M}$) as a function of Tween 20 concentration at pH 5.6 are presented in Fig. 1. Foams exhibited behavior consistent with Tween 20-induced destabilization of the protein foam. The foams demonstrated a sharp decrease in foam stability with 30–100 μM Tween 20. Minimum foam stability was observed at $R = 1.94$ (120 μM Tween). The minimum was followed by a partial restoration of foam stability. This restoration in the foam stability was probably due to the progressive displacement of protein from the interface by Tween 20. A plateau value of stability was achieved at $R = 2.9$. Good agreement is found between the foam stability curves for the Tween 20 and BSA mixture and for Tween 20 alone at $R > 2$. This is confirmatory evidence that foam stability is dominated by Tween 20 at $R > 2$. These data were not normalized because no significant differences in bulk solution conductivity were observed with increasing Tween 20 concentration. The foam stability properties of Tween 20 and BSA mixtures shown in Fig. 1 were evaluated to allow selection of a solution that could be used to assess foam-stabilizing properties of arabinoxylan (Axn). Since it was proposed that Axn may enhance protein functionality by interaction and crosslinking of protein molecules in the adsorbed layer, it was important to select a solution composition where a measurable decrease in foam stability was observed, but insignificant displacement of BSA from

the adsorbed layer by the Tween 20 had occurred. To this end, $R = 0.42$ was chosen and is referred to as the foam test solution at pH 5.6 (FTS-5.6). The sample contained BSA (4.08 mg/mL, $61.8 \mu\text{M}$) and Tween 20 (25 μM).

A small decrease in the bulk conductivity of FTS-5.6 was observed at concentrations of added Axn ≥ 0.2 mg/mL. This coincided exactly with increased viscosity and density of the Axn solutions alone. Figure 2 shows the kinematic viscosity, density, and the bulk conductivity of Axn solutions in concentrations of 0.001–0.6 mg/mL. The increase in viscosity occurred in two phases. The first part of the viscosity curve at 0.001–0.1 mg/mL of Axn showed only a gradual increase in viscosity at Axn concentrations ≈ 0.1 mg/mL. The second part of the curve showed that a significant increase in bulk viscosity occurred at 0.2–0.6 mg/mL of Axn. A significant decrease in bulk conductivity was observed at Axn concentrations corresponding to the observed increase in solution viscosity. Hence, foam stability had to be normalized for changes in the conductivity of the intralamellar liquid.

The normalized foam stability ($\% \Delta C$) for samples of FTS-5.6 containing different concentrations of Axn is shown in Fig. 3. A discrepancy between the normalized foam stability ($\% \Delta C$) and foam stability (C_{300}) curves was observed at ≥ 0.3 mg/mL of added Axn. Nevertheless, the difference in the general appearance of normalized foam stability and foam stability curves as a function of Axn concentration was not considerable and showed similar general trends. The maximum normalized foam stability for FTS-5.6 was observed with 0.2–0.3 mg/mL of Axn. The subsequent decrease in bubble stability at higher Axn concentrations produced lower foam stability values.

The most significant increase in the normalized foam stability of FTS-5.6 was observed at concentrations of < 0.1 mg/mL of added Axn, where no significant increase in bulk viscosity or density was observed. This enhancement of foam stability could derive from interaction of Axn with protein molecules in the adsorbed layer (i.e., restoration of the adsorbed layer integrity through crosslinking of BSA adsorbed at the interface).

Thin Film Drainage

The presence of 0.05–0.1 mg/mL of Axn in FTS-5.6 samples slowed the rate of thin film drainage significantly. The film drainage remained asymmetric in general appearance, but the thin films showed a transformation in the properties of the adsorbed layer. The drainage pattern changed from a mobile, transitional-type drainage pattern characteristic of mixed surfactant and protein stabilized

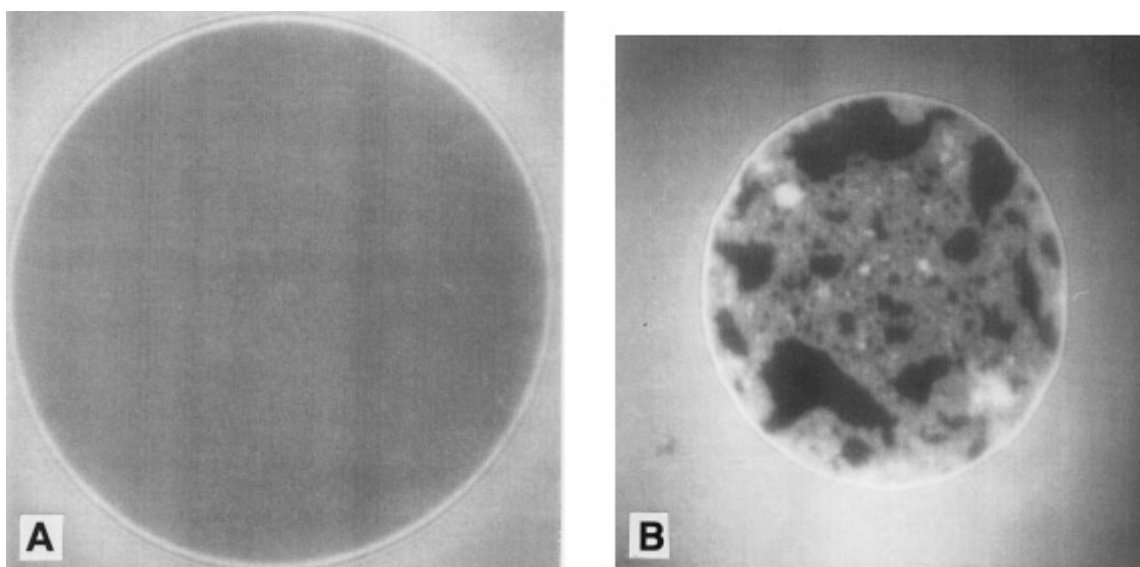


Fig. 4. Equilibrium thin liquid films formed from solutions of foam test solution at pH 5.6 with arabinoxylan at concentrations of 0.05 mg/mL (A) and 0.3 mg/mL (B).

thin film to a highly distorted form of typical protein film drainage. The subsequent equilibrium thin films formed showed significantly increased film thicknesses (Fig. 4a), indicated by pale gray color and regular topography. In the presence of ≥ 0.2 mg/mL of Axn, the initial drainage rate of the thin films of FTS-5.6 appeared to be increased marginally. Pre-equilibrium thin films formed from Tween 20 and BSA solutions that included ≥ 0.25 mg/mL of Axn demonstrated a dual character. Very thin black regions of the thin film coexisted adjacent to thick pale gray regions that formed the vast majority of the film (Fig. 4b). In some instances, a whole range of distinct and stable thin film thicknesses coexisted together within the same film. In general, thin films that contained a highly irregular distribution of thicknesses accounting for >5 – 10% of the planar thin film were unstable.

Equilibrium Film Thickness

The data from thickness measurements performed on thin films stabilized by FTS-5.6 containing a range of concentrations of Axn are presented in Fig. 5. The equilibrium film thicknesses of the thin films was increased considerably with 0–0.3 mg/mL of added Axn, although the thicknesses of thin films at ≈ 0.05 mg/mL of Axn did not appear to vary considerably from those observed for FTS-5.6 in the absence of Axn. A maximum in film thickness was observed with 0.25–0.3 mg/mL of Axn and extended across the vast majority of the film. At higher Axn concentrations, a marked decrease in the thickness of the majority of the film was observed, accompanied by a decrease in film stability. At 0.4 mg/mL of Axn, the film thickness was similar to that observed in the absence of Axn. The stability of the foam at these higher concentrations of added Axn was probably related more to the viscosity of the entrained foam liquid than to interfacial effects.

In the presence of 0.5 and 0.6 mg/mL of Axn, some films were ≈ 10 nm thick, but measurement was difficult because the majority of thin films did not achieve equilibrium film thicknesses before rupture (Fig. 5, dotted line). Concentration ranges are also marked in Fig. 5A and B, where the thin films display a complex appearance. In concentration range A (0.25–0.6 mg/mL of Axn), the thin films of FTS-5.6 contained black regions ≈ 10 nm thick, in a thin film of much greater overall thickness. Film rupture occurred with increasing frequency at higher concentrations of added Axn in FTS-5.6 thin films and is indicated in Fig. 5 by concentration range B (0.3–0.6 mg/mL).

Surface Tension Measurements

The instability of thin films and foams formed from FTS-5.6 at higher concentrations of added Axn (0.3–0.6 mg/mL) may be

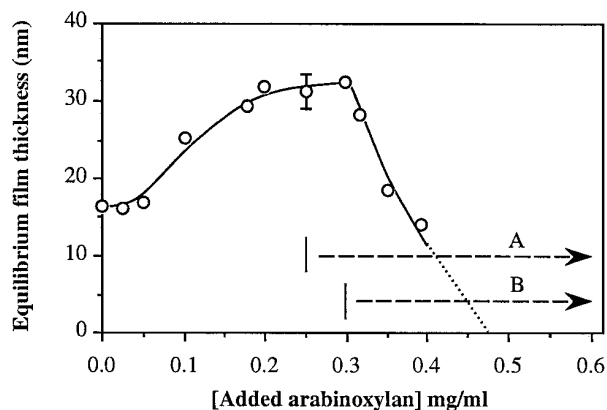


Fig. 5. Effect of arabinosyln on the equilibrium film thickness of thin films formed from foam test solution at pH 5.6. Concentrations of arabinosyln where coexistence of thin black regions in the grey film occurred (A) and where significant levels of film rupture occurred (B). Dotted line indicates the concentration range of arabinosyln over which stable films could not be formed.

partly explained by the surface tension properties of Axn solutions alone. Figure 6 shows the observed reduction in surface tension of Axn solutions in 10 mM citric acid-sodium phosphate buffer at pH 5.6 as a function of concentration. The rate of surface tension reduction for a solution containing 2.5 mg/mL of Axn is shown in the inset. The surface tension was reduced marginally in the Axn concentration range 0.0025–0.05 mg/mL, but at concentrations >2.5 mg/mL, a marked decrease in surface tension was observed. Eliasson and Larsson (1993) reported similar surface active properties for Axn and attributed them to trace amounts of protein associated with the polysaccharide. In general, pseudo-equilibrium surface tension values were not achieved for 2 hr. These data have implications for surface rheological studies performed over extended measurement periods.

Surface Diffusion in the Adsorbed Layer

The lateral diffusion coefficient (D) of DiO-NBD located in the adsorbed layer of thin films formed from solutions of Tween 20 and BSA (123.6 μ M BSA) at $R = 0.46$ as a function of Axn concentration are presented in Fig. 7. The level of disruption to protein-protein interactions in the adsorbed layer ($D = 2.2 \times 10^{-7}$ cm²/sec) observed in thin films from Tween 20 and BSA solutions ($R = 0.46$) was not influenced with 0–25 μ g/mL of Axn. However, with 27–30 μ g/mL of Axn, the adsorbed layers of the thin films rigidify as demonstrated by the abolition of lateral diffusion of the probe molecule. However, lateral diffusion of DiO-NBD was reinstated in 33 μ g/mL of Axn ($D = 1.8 \times 10^{-7}$ cm²/sec) but was considerably reduced from that observed in the absence of Axn. In 40–50 μ g/mL of Axn; lateral diffusion of the probe molecule was restored to levels characteristic for the Tween-destabilized BSA solution indicating the same degree of disruption within the adsorbed layer as was observed in the control system (Tween 20 and BSA alone).

Surface Dilation Measurements

Dilation measurements were performed on 1 mg/mL (15.15 μ M) BSA solutions in a chamber where temperature and humidity were carefully controlled (Fig. 8). The BSA solution was allowed to equilibrate for 18.5 hr and reach equilibrium surface tension, elasticity and viscosity. During the course of this equilibration process, an initial sharp rise was observed in the dilational modulus to a plateau value of ≈ 95 mN/m achieved between 9 and 18.5 hr.

An aliquot of Axn (arrow in Fig. 8) was introduced beneath the interfacial layer to give a final concentration of 5 μ g/mL of Axn in the solution. The bulk fraction was gently but thoroughly mixed, and the dilational measurements were resumed. A significant and almost immediate increase in the elasticity of the interface was observed and developed over several hours following addition of Axn

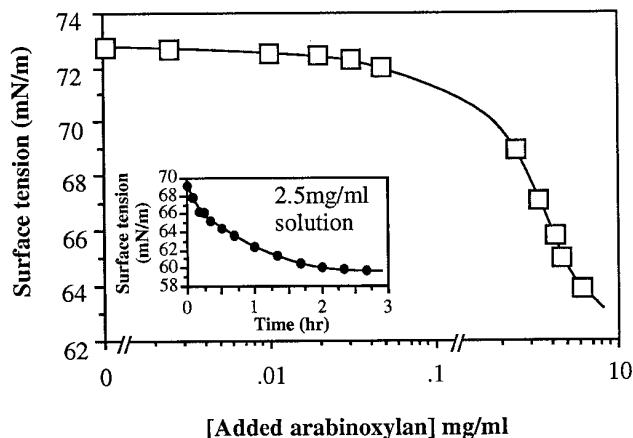


Fig. 6. Surface tension of arabinosyln solutions as a function of concentration (\square) after 20 min of adsorption. Inset: Time dependence (\bullet) of the surface tension for a solution of 2.5 mg/mL of arabinosyln.

to the BSA solution. A second pseudo-plateau in the dilational modulus value was reached ≈ 5 hr after addition of Axn. At the termination of the experiment, 24.5 hr after the Axn had been added, the surface dilational modulus was increased from that of the protein alone by $\approx 22\%$. Only minor variations in the surface tension and surface dilational viscosity were detected during the experiment. The increase in the surface dilational modulus, which consisted mainly of the surface dilational elasticity induced by Axn, was consistent with the results from FRAP and thin film thickness measurements.

DISCUSSION

Food foams are frequently stabilized by proteins that adsorb at air-water interfaces. Reduced stability in many foods results from competitive adsorption of low molecular weight surface active molecules (surfactants-emulsifiers-lipids) that may be present. This contamination produces a decrease in the functionality of the protein and textural-mechanical properties of the foam. This process is observed in a variety of food foams (e.g., beer foam, meringues, doughs) when comparatively small quantities of lipid are present (MacRitchie and Gras 1973) or contaminating surfactant is incorporated during manufacture (Anonymous 1970). Total collapse of the protein-based foam is frequently observed when sufficient contaminating lipid is present. The basis of the instability lies in the two distinct mechanisms that stabilize foams. Proteins stabilize foams by formation of ionic, hydrophobic, and hydrogen bonds, and occasionally covalent disulfide intermolecular interactions (Dickinson et al 1988, Dickinson and Matsumura 1991) which produce a rigid, viscoelastic adsorbed layer or skin surrounding the bubble (Clark 1995). A totally different mechanism of foam film stabilization prevails in surfactant-based foams referred to as the Marangoni mechanism. This relies on the absence of interactions between adsorbed surfactant molecules and the unrestricted migration to regions of high surface tension within the foam film. Thus, surfactant foams are also very stable. However, in the presence of both surfactant and protein, where the protein is the major component, interfacial integrity is compromised as protein-protein interactions in the adsorbed layer are disrupted by the surfactant (Courthaudon et al 1991) causing a reduction in foam stability (Clark et al 1991).

A degree of resistance to surfactant-induced food foam instability can be conferred by altering the physico-chemical properties of both the continuous and disperse phases of the foam. In beer foams or whipped cream, increasing the viscosity of the liquid entrained within the foam can be used to slow down the rate of liquid drainage from the foam. This is important because slower drainage results in thicker, more stable foam films and counteracts the observation

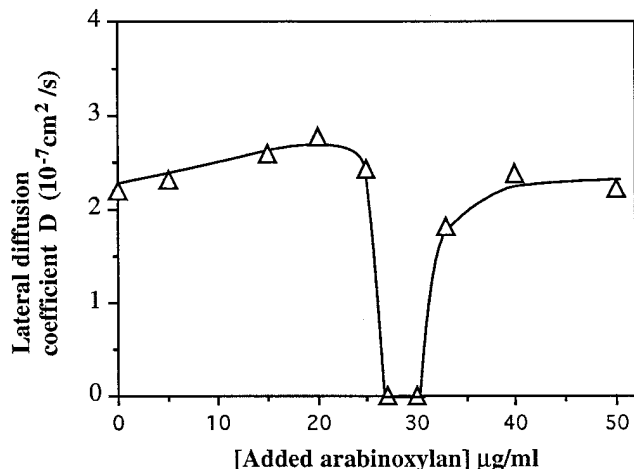


Fig. 7. Effect of arabinoxylan concentration on the surface lateral diffusion of DiO-NBD in the adsorbed layer of thin films formed from Tween 20 and BSA solutions (molar ratio $R = 0.46$).

that liquid films composed of protein and surfactant mixtures tend to drain much more rapidly than liquid films formed from protein alone (Clark et al 1991). In beer foam and ice cream, polysaccharides are often added to the solution to increase bulk (continuous phase) viscosity among other effects and, hence, increase foam stability (Anonymous 1970, Vincent and von Husby 1990).

Alongside the use of polysaccharides in food foams as viscosity enhancing agents, there is some evidence that supports an additional role involving interaction between the polysaccharides and other molecules, particularly proteins in the adsorbed layer (Anonymous 1970; Vincent and von Husby 1990; Dickinson 1993, 1994). The formation of polysaccharide and protein complexes (Stainsby 1980) has been associated with enhancement of the interfacial properties of proteins and foam or emulsion stabilization (Anonymous 1970, Stainsby 1980) by stiffening the interfacial layer and reducing the liquid drainage from thin films. These properties of wheat pentosans complement other properties such as enhancement of the gel-like properties of doughs (flour foam matrix) in confectionery manufacture (Izydorkzyk and Biliaderis 1993, Gruppen et al 1993a) and involvement in crosslinking (Fry 1983) within plant cell walls.

The work described in this article aimed to examine the foam stabilizing properties of Axn fractions in a surfactant and protein mixture that produced foams of defined, reduced foam stability, and explored the possible interaction between Axn and proteins in the adsorbed layer under conditions representative of those found in baked products. To this end, a foam test solution (FTS-5.6) of Tween 20 and BSA was mixed at a molar ratio of $R = 0.42$ and the foaming, thin film, and surface properties were examined as a function of Axn concentration at pH of 5.6, which was representative of that found in bread dough.

The foam stability of FTS-5.6 increased significantly with Axn up to a total concentration of 0.2 mg/mL of added Axn (Fig. 3). However, it was initially unclear whether this effect derived from Axn-induced increases to bulk viscosity or rigidification of the adsorbed layer through interactions with adsorbed proteins. The contribution of Axn to bulk viscosity was assessed by measurement of the kinematic viscosity. This measurement of viscosity was chosen because the geometry of the measurement (timing of the flow of liquid through a calibrated capillary) relates most closely to thin film drainage. In addition, it has been reported from studies of poly-

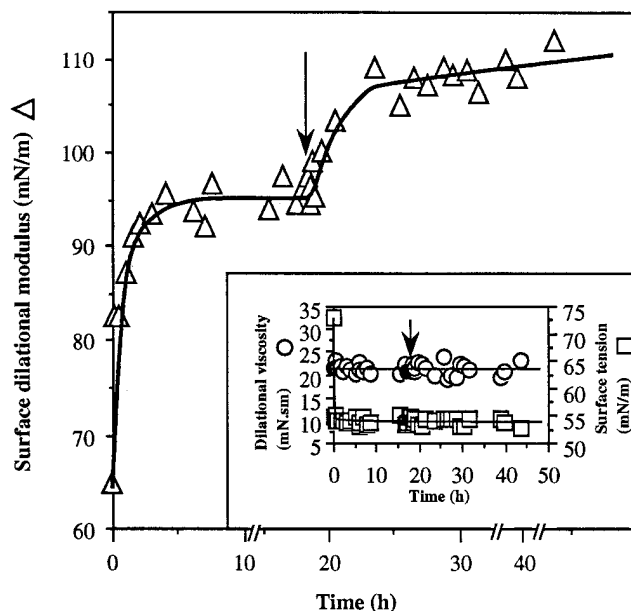


Fig. 8. Time-dependent surface dilational modulus (Δ) of 1 mg/mL of bovine serum albumin (BSA). Arrow indicates addition of 5 $\mu\text{g/mL}$ of arabinoxylan. Inset: surface tension (\square) and surface dilational viscosity (\circ) under the same conditions.

saccharides at low concentrations that kinematic viscometry is a suitable technique for determining minor changes in solution viscosity (Jackson et al 1980, Vincent and von Husby 1990). The results show that only minor increases in kinematic viscosity occur in solutions containing up to 0.1 mg/mL of Axn, and this concentration range correlates with the most significant observed increases in foam stability. This result is consistent with the initial increase in foam stability involving Axn exerting an effect on the adsorbed layer.

The hypothesis that this effect involved Axn-mediated cross-linking of proteins in the adsorbed layer is supported by data from film drainage, film thickness determinations, measurements of molecular diffusion in the adsorbed layer, and surface dilation studies. The reduction in drainage rate and pattern from asymmetric to symmetric is consistent with rigidification of the adsorbed layer brought about by multivalent interactions between adsorbed protein and Axn. This would result in a build-up of material in the adsorbed layer and is congruous with the measured increase in equilibrium foam film thickness (Fig. 5), which effectively is doubled with 0.2–0.3 mg/mL of Axn. Axn-mediated crosslink formation within the adsorbed layers of the foam film would create barriers to lateral diffusion of adsorbed molecules as the network developed. This correlates directly with the reduction and abolition of diffusion of the probe molecule as determined by FRAP experiments (Fig. 7). Note that abolition of diffusion was observed under conditions where the component concentrations and molar ratio ($R = 0.46$ rather than 0.42) produced more significant breakdown of protein-protein interactions than in FTS-5.6. Such observations compare favorably with previously reported observations of foam and thin film stabilization by polyphenol-mediated crosslink formation (Sarker et al 1995). Conclusive proof of the association of Axn with the adsorbed layer comes from the surface dilation experiment. Here, a very low concentration of Axn was added to the solution of BSA alone following formation and equilibration of the BSA adsorbed layer. The experiment is crucial for a number of reasons. First, the low concentration of Axn added was insufficient to measurably alter the bulk viscosity of the sample and thus decouples bulk and surface effects. Second, the subsequent increase in surface dilational modulus following addition of Axn confirms that previous observations involving changes in adsorbed layer properties in foam test system thin films did not involve a secondary effect, such as specific sequestration of Tween 20 by the polysaccharide. Finally, the time-dependent increase in the surface dilational modulus following addition of the Axn is consistent with development of interactions in the adsorbed layer mediated by crosslinks.

The increase in foam stability due to crosslinking occurred when the concentration of added Axn in the solutions used to form thin films of FTS-5.6 was 0.05–0.1 mg/mL. At higher concentrations (≥ 0.3 mg/mL of Axn), the thin films showed two or more distinct thicknesses within the same film (Fig. 4B) and an increase in the number of adsorbed aggregates, these foam films were almost always unstable. It is likely that the interaction of the polysaccharides with protein in the bulk solution caused a depletion of monomer protein available for adsorption, possibly by a mechanism involving sequestration of the protein by the Axn. Thus, a reduced concentration of protein available to adsorb at the interface would lead to a greater proportion of adsorbed Tween 20 and hence a less rigid (surfactant-like), thinner film. The bulk viscosity increase did not appear to restore foam stability in Axn and FTS-5.6 systems at >0.3 mg/mL of Axn because the overall thickness of foam films was reduced considerably, and at Axn concentrations of 0.5–0.6 mg/mL, equilibrium thin films could not be formed.

The BSA-Axn interactions at the interface give rise to high concentrations of Axn near the adsorbed layer, as evidenced by the increase in foam film thickness (Fig. 5). This may lead to an increase in bulk viscosity adjacent to the adsorbed layer, much greater than that measured (Fig. 2), which in turn could slow down the rate of drainage from foam lamellae and increase foam stability.

However, this local viscosity change could not increase the surface modulus (Fig. 8) or immobilize the adsorbed layer (Fig. 7), suggesting that although local viscosity effects could significantly contribute to improving foam stability, the principle mechanism involved in the system studied here is the interaction between Axn and BSA to restore the integrity of the adsorbed layer.

Little information exists on the interaction between Axn and milk proteins but interaction between Axn and plant cell wall material (Markwalder and Neukom 1976, Fry 1983, Rybka et al 1993) and proteins in dough (Michniewicz et al 1991, Gruppen et al 1993b) has been observed. The nature of this interaction is thought to involve oxidative coupling of the ferulic acid (0.05%/Axn molecule) phenolic residues, randomly located along the Axn backbone, to other phenolic compounds located in neighboring protein molecules or cell wall polysaccharides (Fry 1983, Moore et al 1990). However, hydrophobic interaction and hydrogen bonding are more likely to have occurred between the Axn and the protein in this instance and could account for the significant increase in foam stability and film thickness along with the rigidification of the adsorbed layer confirmed by FRAP and dilational rheology measurements.

Interaction between Axn and BSA was evidenced not only in measured properties but also in the formation of aggregates. The Tween 20 and BSA foam test system clearly indicated crosslinking had taken place. The poor but measurable surface activity of Axn may have contributed to foam instability observed at higher polysaccharide concentrations, due to competitive adsorption by the polysaccharide. If much of the protein had been crosslinked resulting in surface and solution aggregation then adsorbed polysaccharide may not have been sufficient to support stable thin films. The protein depletion mechanism and poor surface activity of the polysaccharide may behave in an additive manner which is responsible for the film rupture observed, despite higher intralamellar viscosities.

CONCLUSIONS

Evidence has been presented that supports the hypothesis that Axn can stabilize protein foams not only by increasing the viscosity of interlamellar liquid and altering the drainage properties of the foam films, but by the mediation of interactions and crosslinks between proteins in the adsorbed layer. The latter is supported by observations of rigidification of the adsorbed layer, increased equilibrium film thickness of adsorbed layers, the formation of networks in the adsorbed layer which impede surface lateral diffusion and an increase in the surface dilational modulus in the presence of Axn.

These results provide an insight into the role of arabinoxylans in foam stability in a number of food products. Thus, the basis of the increase in loaf volume (Hoseney et al 1969) associated with the presence of pentosan may reside in the crosslinking of flour proteins by Axn. The data reported here shows that a degree of foam stability may be conferred to protein-based foams against the disruptive effects of competitive adsorption by low molecular weight surfactants in the presence of polysaccharides that possess crosslinking capability.

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