Lipid-Protein Interactions in Beer and Beer Foam Brewed with Wheat Flour[1]

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ABSTRACT

Use of wheat flour as a brewing adjunct has been shown to enhance beer foam stability, as measured by the Rudin method, and also to give protection against the adverse effects of added triolein on foam stability. The efficiency of this protective action is dependent on the contact time between the lipid and the beer and is specific to triolein. The addition of palmitic acid showed different effects on the foam stability of beer brewed using wheat flour. This effect was postulated to be caused by the specific binding of triolein to proteins derived from wheat flour. Interaction between triolein and beer proteins was identified by precipitation of radiolabeled lipid with trichloroacetic acid. Use of the detergent n-octyl-β-D-glucopyranoside allowed a semiquantitative estimation of the affinity of soluble proteins for triolein. Using this method, it was found that there is no significant difference in the affinity of triolein for proteins derived from beers brewed from either an all-barley malt grist or one containing wheat flour. It was concluded that lipid-protein interactions exist in beer, but their affinity is too low to allow the identification of the proteins responsible.

Key words: Wheat flour, Foam stability, Lipid-protein interactions, n-Octyl-β-D-glucopyranoside.

Lipids in beer can affect a number of qualities of the product, one of which is the foam stability. The effect of lipid material on beer foam stability has been a matter of much research. Whereas it is generally agreed that lipids will destabilize beer foam stability by reducing the surface tension of the liquid film around the gas bubbles, it is thought by some researchers that the endogenous levels of lipid classes, e.g., triglycerides or fatty acids, are usually too low to have any significant effect (4). However, it has also been demonstrated that adding fractions of malt lipid extract at levels of 1.0 mg/L reduces foam stability by up to 62%. A combination of triglyceride and phospholipid fractions is the most effective (3). Under well-controlled production conditions, the low levels of lipids surviving the finished product will have no significant effect on foam stability. However, if precautions are not taken to minimize wort lipid levels, beer foam stability is likely to be adversely affected (3).

Another aspect of the destabilization of beer foam by lipids was investigated by Roberts et al (8). It was found that the extent of the reduction in beer foam stability, as measured by the Rudin method (9), was dependent on how long the lipid had been in contact with the beer. The shorter the contact time, the greater the effect. It was proposed that lipids exist in either of two physical states: an initial state where the lipid is in a transitory micellar form and not associated with any other components of beer, and a final state, where after a time, individual lipid molecules leave the micelles and associate with beer proteins. In the initial state, the micellar lipids are potent foam destabilizers, but when associated with proteins their effect is reduced.

Incorporation of wheat flour into the grist is known to enhance foam stability of beer (5) as well as improving other aspects of beer quality (shelf life and stability to chill haze). This has been attributed to an increase in the levels of high molecular weight glycoproteins, although little work has been done to identify which proteins of wheat flour are responsible.

In the modern process of making bread, wheat flour is mixed with shortening fat and water and then subjected to a high-energy mixing process to form a stiff dough. If this process is carried out under an atmosphere of nitrogen gas there is a significant increase in the extractable bound lipid fraction, which has been shown to be mainly caused by the nonselective binding of triglycerides by gluten proteins (1). Subsequent work by Frazier et al (2) involved using radiolabeled triolein to follow the fate of triglycerides during dough development under nitrogen. A significant proportion of the lipid was found to be stably bound to a protein derived from the acetic acid-soluble fraction of dough. The protein, named ligolin, has a molecular weight of approximately 9,000–11,000 daltons and is speculated to be of fundamental importance in the formation of the ideal dough structure.

The objective of the research presented here was to determine if ligolin is active in binding triglycerides in beers brewed with wheat flour, and if so, if such interactions are involved in the enhancement of beer foam stability.

EXPERIMENTAL

Brewing of Beers

Beers were brewed from a grist of either 100% milled barley malt or one containing 15% whole wheat flour as used in commercial brewing. Water treated with various inorganic salts (Burtonized) was added to the grist in a ratio of 1.1 L per 500 g of grist at a temperature of 75° C. Mashing was continued for 60 min at 65° C with mechanical stirring. Wort separation was by lautering and involved sparging with brewing liquor at 75° C until the runnings were below 1.005° specific gravity. The sweet wort was boiled with whole hops (1 g/L) for 90 min, allowed to cool, and carefully decanted from the trub. The original gravity and volume were measured. Fermentation was carried out at 18° C, using a commercial brewing yeast at 2.6 g/L, until the specific gravity was below 1.010°. Post fermentation treatment involved cold conditioning at 4° C for four days to promote yeast sedimentation and filtration through sterilizing grade filter pads under mild reduced pressure.

Measurement of Foam Stability

The Rudin method (9) was used to measure beer foam stability with carbon dioxide as the foaming gas. Before use, beers were degassed by gentle stirring for 2 hr and then brought to 20° C, at which temperature foam stability was measured. Results are quoted as a head retention value (HRV) in seconds.

Foam Studies

The effect of adding either palmitic acid or triolein (both from Sigma Chemical Co., Dorset, England) on the foam stability of both wheat flour beer and all-malt beer was investigated.

The Effect of Lipid Concentration on Foam Stability

Triolein was dissolved in propan-2-ol at a concentration of 10 mg/ml. Volumes of this solution were added to stirred samples of each beer (350 ml) so that the final lipid concentration ranged from 0 to 23 mg/L. The treated beers were stirred for 60 min, after which time the HRV was determined. In addition, propan-2-ol (0.8 ml) was added to a sample of each beer in order to assess the effect of the solvent on foam stability. This volume is equivalent to the maximum volume of triolein in propan-2-ol used in this experiment.

Similarly, palmitic acid dissolved in ethanol (3.5 mg/ml) was added to samples (350 ml) of each beer so that its final

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concentration ranged from 0 to 20 mg/L. The beers were treated in the same way as described above and their HRV determined. The effect of ethanol (2 ml) on foam stability was also assessed.

The Effect of Contact Time on Foam Stability

Triolein in propan-2-ol (10 mg/ml) was added to stirred samples of each beer so that its final concentration was 23 mg/L and stirred for a period of time ranging from 10 to 180 min. The HRV of each sample was then determined.

With palmitic acid, a different method was used to assess the effect of contact time of the fatty acid on foam stability. It was found in preliminary experiments that the HRV of a beer sample that had been in contact with palmitic acid (20 mg/L) for 10 min increased over the time taken to perform the replicate analyses. Therefore, palmitic acid in ethanol was added to samples of each beer (350 ml), so that its final concentration was 20 mg/L, and stirred for 5 min. A volume of beer sufficient to perform one HRV analysis was taken, and the time at which the test started was recorded. This was defined as the contact time. Subsequent volumes of treated beer were taken at different times and the HRV measured. In order to gain replicate values, the whole experiment was repeated using a fresh sample of the same beer stock.

Interaction of Radiolabeled 1-^{14}C-Triolein with Beer Foam Proteins

To investigate possible interactions between lipids and beer proteins, radiolabeled 1-^{14}C-triolein was chosen because of its known ability to bind to certain wheat flour proteins (2).

Preparation of Radiolabeled 1-^{14}C-Triolein Stock Solution

Radiolabeled 1-^{14}C-triolein (Amersham International, Bucks, England) was redissolved in propan-2-ol (10 ml) after the original solvent had been removed with nitrogen gas. Nonradiolabeled triolein in propan-2-ol (10 mg/ml) was used as supplied (Sigma Chemical Co.)

A stock solution of triolein was prepared by mixing radiolabeled lipid in propan-2-ol (0.3 ml), nonradiolabeled triolein (1.0 ml), and making the volume to 25 ml with propan-2-ol. The specific activity of this stock solution was 0.15 nCi/(μg/ml).

Radioactivity of samples was measured by liquid scintillation counting. The scintillation cocktail contained 2,5-di-phenyloxazole and 1,4-di-2-(5-phenyloxazolylbenzene) at 10 and 0.3 g, respectively, per 2.5 L of toluene. This solution was mixed with Triton-X-100 in a volume ratio of 2:1. Samples were mixed with 10 ml of the scintillation cocktail. Counting efficiencies were determined automatically by the external standardization ratio method. Counting times were either 2 or 10 min depending on the activity of the sample being assayed.

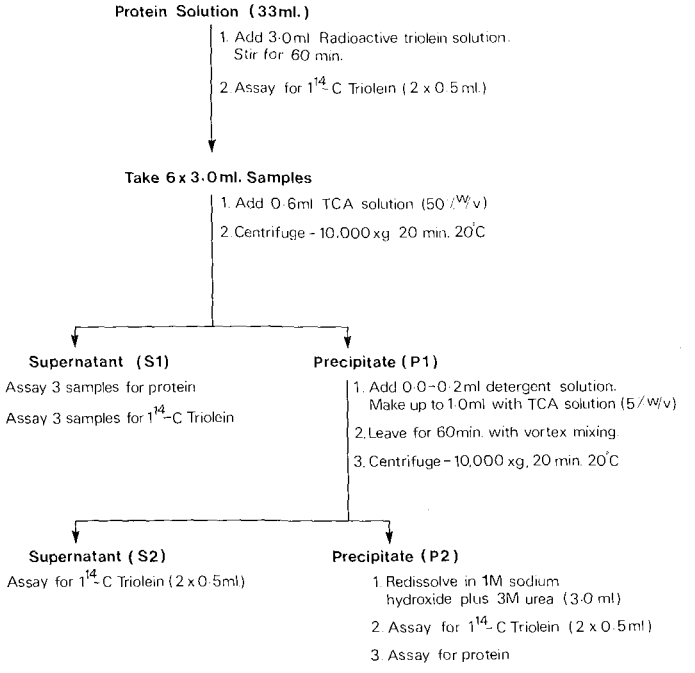

Fig. 1. Procedure for evaluating affinity of protein solutions for triolein.

Delipidation of Protein Solutions Using n-Octyl-β-D-Glucopyranoside

Figure 1 summarizes the procedure used to investigate the relative affinity of various protein solutions for triolein.

The detergent n-octyl-β-D-glucopyranoside (Sigma Chemical Co.) was dissolved in deionized water at a concentration of 1.14*M*. Protein solutions were adjusted to a concentration of 1 mg/ml by dilution with an appropriate solvent (see below). Therefore, the same weight of protein was used in every test. The method described by Millar (7) was used to assay for protein and is a modification of the method of Lowry (6).

Six aliquot samples (3.0 ml) were removed from the incubated protein solution and precipitated with 0.6 ml of a 50% w/v trichloroacetic acid (TCA) solution. Three of the six supernatant (S1) fractions were assayed for protein, and the other three were used to determine the amount of nonprecipitated radiolabeled lipid by liquid scintillation counting. The precipitates (P1) were washed with increasing concentrations (0–225 μmol) of a solution of the detergent in 5% w/v TCA for 60 min with frequent vortex mixing. After centrifuging at 10,000 × *g* for 20 min at 20° C in an angle head rotor, the supernatant (S2) was removed and assayed for 1-^{14}C-triolein. The remaining precipitate (P2) was redissolved in 3.0 ml of a solution of sodium hydroxide (1.0*M*) and urea (3.0*M*) and assayed for 1-^{14}C-triolein and protein.

Cereal Proteins

Samples (20 g) of milled barley malt and wheat flour were extracted with 0.5*M* aqueous sodium chloride (80 ml) and then with 0.1*M* aqueous acetic acid (80 ml) at ambient temperature. Each flour sample was mixed by vigorous stirring for 60 min with 80 ml of the sodium chloride solution. The extract was centrifuged at 8,000 × *g* for 20 min at 20° C. This was repeated twice and the extracts were pooled. The residual solids were then extracted with acetic acid (80 ml) for 60 min and centrifuged under the same conditions. The second acidic extraction was left stirring overnight in the same volume of acetic acid. After centrifuging, a third extraction was carried out for 120 min, again in 80 ml of solvent. The three extracts were pooled, centrifuged at 23,000 × *g* for 20 min at 20° C and assayed for protein. The extract was diluted to a protein concentration of 1 mg/ml using 0.1*M* acetic acid and then incubated with radiolabeled triolein as outlined in Figure 1.

Beer Foam Proteins

Foam was generated from beers brewed with and without wheat flour by injecting oxygen-free nitrogen gas into 150 ml of each beer. The foam generated was guided up a vertical glass tube 1.6 m × 2.5 cm in order to drain the excess liquid. The foam was collected at the top of the column until no more stable foam could be generated.

The collapsed foam was assayed for protein after membrane filtration (0.45 μm) to remove solid material. Collapsed foam that had been filtered was adjusted to a protein concentration of 1 mg/ml with glass-distilled water and then incubated with radiolabeled triolein as described in Figure 1.

RESULTS AND DISCUSSION

The Effect of Lipid Concentration on Beer Foam Stability

The results presented in Table I show the effect of increasing concentrations of triolein and palmitic acid on the foam stability of beers brewed with and without wheat flour. At zero concentration

of both lipids, the stability of wheat flour beer foam was significantly greater than that of the all-malt beer. This is consistent with the results of Leach (5). Increasing the concentration of triolein has a negative effect on the foam stability of both beers. However, the reduction in HRV from control values (ΔHRV) is less for wheat flour beer, especially at the higher concentrations of triolein. There was no significant increase in HRV of both beers due to either lipid solvent alone. The influence of palmitic acid on foam stability is less evident. There was very little effect on the HRV for all-malt beer even at a concentration of 20 mg/L, whereas the foam stability of the wheat flour beer was reduced by 3–5 sec.

The Effect of Contact Time on Foam Stability

For both lipids, the reduction in HRV from control values decreased as the contact time increased (Table II). This was seen for both beers and is consistent with findings of Roberts (8). However, the rate of recovery of foam stability was different for each lipid-beer combination. With triolein, the rate of recovery of the head retention value of wheat flour beer was faster than for all-malt beer. After 10 min contact time, the wheat flour beer had an HRV that was 88.8% of the control value. After the same contact time, the all-malt beer had recovered 82.6% of its control value. With the precision of the measurement being typically ± 1.5 sec, this is a significant difference. The same pattern was seen after 60 min contact time. When palmitic acid was added to all-malt beer, there was a rapid recovery of HRV, 92.2% of the control value being reached after only 22 min. This was not seen with wheat flour beer, where there was a larger reduction in foam stability (71.7% of the control value) after a contact time of 5.5 min and a slow HRV recovery rate.

These findings help to explain the small changes in foam stability of all-malt beer when the concentration of palmitic acid was increased (Table I). The incubation time of 60 min was too long to demonstrate any effect of the palmitic acid on foam stability of all-malt beer. However, the initial reduction in HRV of wheat flour beer caused by palmitic acid at 20 mg/L was great enough for there to still be a measurable effect after this period of contact.

There were significant differences in the ability of the two beers to tolerate the destabilizing action of both triolein and palmitic acid. Wheat flour beer was less affected by increasing levels of triolein and overcame these effects more quickly in a quicker time. In contrast, adding palmitic acid to wheat flour beer caused a greater drop in foam stability when compared to all-malt beer. Also, this beer was less able to overcome the negative action of the fatty acid on foam stability. These data are consistent with the model proposed by Roberts (8) and indicate that there may be specific interactions between triolein and components derived from wheat flour that do not occur in all-malt beer.

Interaction of Radiolabeled 1-^{14}C-Triolein with Protein Solution

The sodium chloride extracts of barley malt and wheat flour contained 625.9 ± 75.4 mg and 339.5 ± 9.1 mg of protein, respectively. These values represent 33.3% and 19.9% of the total protein present in 20 g of milled cereals. Equivalent figures for the acetic acid-soluble proteins of the two cereals are 310.4 ± 4.4 mg (20%) and 714.5 ± 26.5 mg (42%). Wheat flour had a significantly

TABLE I
The Effect of Lipid Concentration on Beer Foam Stability (HRV)

Concentration (mg/L)	All Malt Beer		Wheat Flour Beer	
	HRV (sec)[a]	Δ HRV[b]	HRV (sec)[a]	Δ HRV[b]
Triolein				
0	106.3 ± 1.2	···	113.0 ± 1.1	···
5	103.8 ± 1.3	−2.5	109.1 ± 0.6	−3.9
10	97.5 ± 1.0	−8.8	108.0 ± 2.2	−5.0
20	95.6 ± 1.1	−10.7	109.3 ± 1.0	−3.7
Propan-2-ol (0.8 ml)	108.9 ± 1.5	+2.6	114.0 ± 2.1	+1.0
Palmitic acid				
0	103.6 ± 1.1	···	111.0 ± 1.5	···
5	104.0 ± 2.0	+0.4	105.3 ± 1.2	−5.9
10	102.4 ± 2.2	−1.2	108.1 ± 1.0	−3.1
20	103.8 ± 0.9	+0.2	108.1 ± 0.9	−3.1
Ethanol (2 ml)	ND[c]		113.5 ± 1.3	+2.3

[a] HRV = Mean head retention value ± standard deviation of four replicates.
[b] ΔHRV = HRV (zero lipid) − HRV (mg/L lipid).
[c] ND = not determined.

TABLE II
The Effect of Triolein and Palmitic Acid Contact Time on Beer Foam Stability (HRV)

Contact Time (min)	% Control HRV[a]	
	All Malt Beer	Wheat Flour Beer
Triolein (23 mg/L beer)		
0	100.0	100.0
10	82.6	88.8
60	89.9	95.3
180	96.2	98.8
Palmitic acid (20 mg/L beer)		
0	100.0	100.0
5.5	80.9	71.7
11.0	89.4	76.5
16.5	92.2	82.3
22.0	92.2	84.1

[a] HRV = Head retention value in seconds.

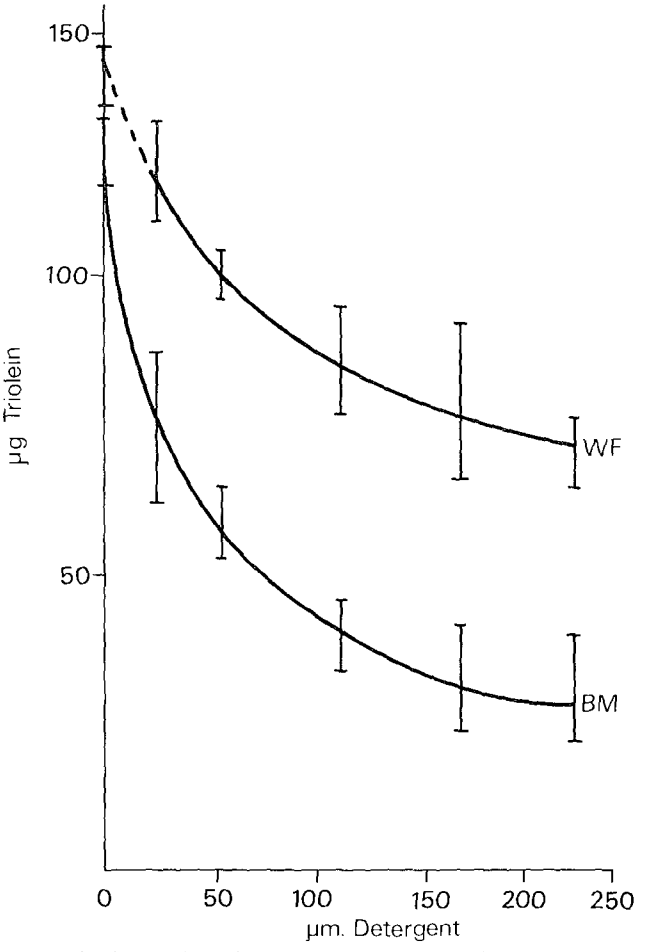

Fig. 2. Triolein remaining in trichloroacetic acid-precipate fraction after washing with n-octyl-β-D-glucopyranoside. Acetic acid-soluble fractions of wheat (**WF**) and barley malt (**BM**).

higher level of acid-soluble proteins, which is indicative of its unique ability to form dough structures.

Figure 2 shows the weight of triolein, as calculated from the specific activity of the radiolabeled lipid solution, remaining in the TCA-precipitated fraction of the acetic acid-soluble extracts of wheat flour and barley malt after washing with increasing concentrations of n-octyl-β-D-glucopyranoside (fraction P2, Fig. 1). The graphs are plotted through the mean value of three determinations, and the bars show the range of results.

At zero concentration of detergent, some 90% of the total triolein present (137 μg) was precipitated from both acetic acid extracts by TCA, and it was therefore concluded to be associated to protein. Of the total protein present (2,750 μg) in the 3.0-ml test sample, 1,099.2 ± 65.7 μg of barley malt proteins were precipitated by 7% w/v TCA in the absence of detergent. The equivalent figure for the wheat flour extract was 2,331.0 ± 198.5 μg. The lesser amount of protein precipitated from the barley malt extract may reflect a lower molecular weight of the acetic acid-soluble proteins (TCA is less efficient at precipitating certain proteins, such as insulin).

At a detergent concentration of 225 μm, the mean weights of triolein remaining in the resolubilized TCA precipitate fractions (P2) of barley malt and wheat flour extracts were 27 and 69 μg, respectively. Observing the range of these results (Fig. 2), these values indicate a significantly higher affinity of the wheat flour proteins for triolein. The weight of protein remaining in this fraction after detergent washing (225 μm) was found to be 552.0 ± 7.7 μg (50.2% of the total precipitated protein) for the barley malt extract and 2,012.9 ± 48.9 μg (86.4%) for the wheat flour extract. It is assumed that there was significant resolubilization of TCA precipitated protein by n-octyl-β-D-glucopyranoside. This could not be confirmed by assaying the detergent supernatant fraction S2 (Fig. 1), because it is incompatible with the reagents used in the protein assay. It is probable that a concentration of TCA higher than the 5% w/v used would keep the precipitated proteins out of solution during detergent washing.

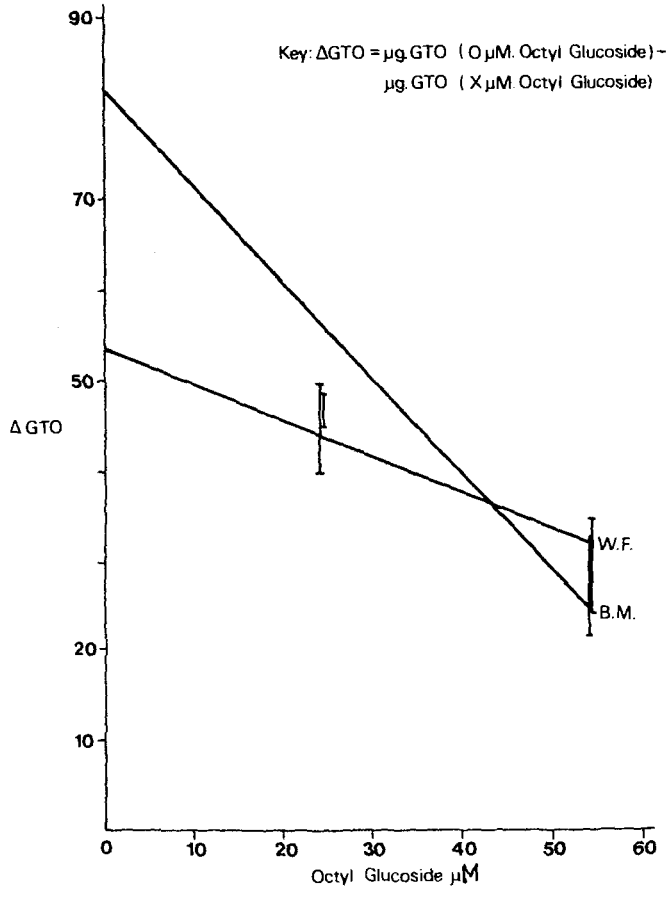

Fig. 3. Removal of triolein from acetic acid-soluble fraction of wheat (**WF**) and barley malt (**BM**) with detergent concentration.

Overall recoveries of radiolabeled triolein were 75–100% and typically greater than 90%.

The resolubilization of protein, and therefore possibly lipid-protein complexes, at high detergent concentrations, and also the likely heterogenous nature of the precipitated protein, prevents any determination of molar binding ratios, and in turn prevents direct comparison of the affinity of the two protein extracts for triolein. However, over the detergent concentration range 0–57 μ*M* there is minimal resolubilization of protein from either extract, i.e, 96.4 to 86.4% of total precipitated wheat flour proteins and 83.7 to 89.2% of total precipitated barley malt proteins. Therefore, the rate of loss of radiolabeled triolein from fraction P2 (Fig. 1) can be taken as a measure of the affinity of the protein extracts for the lipid. The weight of triolein, abbreviated to GTO (glyceryl trioleate), remaining in fraction P2 at 225 μ*M* detergent is assumed to be stably bound and insensitive to the concentrations of detergents used in this experiment. It is therefore subtracted from the values at 0, 25, and 57 μ*M* detergent to give a ΔGTO value, defined as μg GTO (0 μ*M* octylglucoside) − μg GTO (*x* μ*M* octylglucoside). The resulting graphs are shown for the barley malt and wheat flour acetic acid-soluble proteins in Figure 3. Straight lines were obtained for each set of data, and the regression coefficients (Table III) were statistically significant. It is therefore valid to take the slope of the line as a measure of the rate of removal of triolein from the protein fraction and its inverse as an estimate of the affinity. The resulting *K'* (affinity) values are shown in Table III. By joint regression analysis, the *K'* values for the two cereal extracts are significantly different ($P < 0.001$). Therefore, the acetic acid-soluble proteins of wheat flour show a higher affinity for triolein than the equivalent extract of barley malt. This is to be expected, because the equivalent fraction of wheat flour dough proteins has been shown to bind triglycerides during dough development (1,2). The higher stability of the ligolin-triolein complex isolated from dough (2) is assumed to result in part from the conditions used to develop the dough, namely a nitrogen atmosphere and a high energy input.

The requirement for a nitrogen atmosphere to isolate ligolin was the reason this gas was used to generate beer foams. Equivalent affinity data were generated for the two beer foam protein fractions. Table IV shows that there is little difference in the protein content of the two beers as measured using alkaline-copper reagent. It must be noted that this method assays on the basis of aromatic amino acids, and the results will not necessarily reflect

TABLE III
Affinity Values and Regression Coefficients for Protein Fractions

Protein Fraction	Affinity Value *K'*	Linear Regression Coefficient
Wheat flour acetic acid extract	2.47	−0.91
Barley malt acetic acid extract	0.93	−0.94
Wheat flour beer foam	1.69	−0.91
All-malt beer foam	1.32	−0.91

TABLE IV
Protein Levels in Beer and in Beer Foam[a]

	mg Protein		
Beer Type	Beer	Collapsed Foam	Membrane-Filtered Foam
All malt beer	536.3 ± 15.9	415.0 ± 6.6	330.4 ± 1.7
Wheat flour beer	521.3 ± 26.5	368.6 ± 22.0	358.4 ± 15.8

[a] Results are mean ± standard deviation of duplicate experiments.

any differences in the levels of high molecular weight proteins in the two beers. The protein contents of the foams before and after membrane filtration show a greater amount of insoluble protein in the fraction derived from all-malt beer.

TCA precipitates lipid-protein complexes from foam protein fractions derived from both beers, 46 μg of the lipid being precipitated with wheat flour beer foam proteins and 48 μg from all-barley-malt beer foam proteins.

A higher level of protein is present in the P2 fraction of wheat flour beer foam at all detergent concentrations—between 798.8 $\pm$ 47.7 (at 0 μM detergent) and a minimum of 510.0 $\pm$ 84.9 (at 25 μM detergent) compared to 270 μg (0 μM detergent) and 418.5 μg (171 μM detergent) for the barley malt beer foam fraction.

This difference shows a higher degree of solubility of barley-malt beer foam proteins in the presence and absence of detergent and could be caused by differences in the physical nature of the proteins, carbohydrate content and hydrophobicity. Over the detergent range 0–57 μM there is again minimal resolubilization of both protein fractions. There is no significant difference in the K' values for either foam protein fraction (Table III), indicating that there is no preferential affinity of wheat flour beer foam proteins for triolein under the conditions of this experiment.

CONCLUSIONS

The use of wheat flour was shown to protect the foam stability of the finished beer from the adverse effects of added triolein, but had no such effect when stressed with palmitic acid.

Lipid-protein interactions were shown to exist in beers brewed with and without wheat flour using TCA to precipitate such complexes; however, there was no significant difference in the affinity of wheat flour beer foam proteins and all-malt beer foam proteins for triolein as determined using n-octyl-β-D-glucopyranoside. The acetic acid-soluble proteins of wheat flour did show a degree of affinity for triolein, but no evidence was found that they are active in wheat flour beer. The stability of triolein-protein complexes present in either beer was concluded to be very low. Their effect on foam stability is therefore probably minimal.

It is possible that other characteristics of wheat flour proteins present in beer, such as hydrophobicity, solubility, or carbohydrate content are more important factors in influencing foam stability and overcoming the adverse effects of high levels of triolein.

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