Egg Albumen as a Source of Foam Polypeptide in Beer[1]

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ABSTRACT

Egg white (albumen) can be used to improve the foam characteristics of beers. Hydrolysis increases the foam potential of albumen to a level considerably superior to that of beer polypeptides and commercial whipping proteins from soy beans and also removes any tendency to cause hazes in pasteurized beer. It appears, however, that unhydrolyzed albumen is able to improve beer head in ways additional to acting as a simple source of protein. Addition of hydrolyzed albumen can be used as a diagnostic test for poor foams through its effect on the head retention value of beers ultrafiltered to remove material of molecular size greater than 1,000. Hydrolyzed albumen has very little ability to protect beer foam from the deleterious effect of lipid, yet it does provide the polypeptide backbone to those beers that lack sufficient foam protein, for example, those of low original gravity, those encountering excessive degrees of proteolysis, and those incorporating high levels of sugar adjuncts. If a beer already contains sufficient foaming polypeptides, then its head will not be dramatically improved by hydrolyzed albumen. However, as no simple test exists for the quantitation of foaming polypeptides in beer, the inclusion of hydrolyzed albumen provides assurance that a beer will contain sufficient of these materials.

Key words: Albumen, Hydrolysis, Foam parameters, Analysis of beer foam

Diverse factors influence the quality of the head that forms on beer as it is dispensed (Table I). Such factors include the quantity and type of gas that is present in the beverage, the shape and cleanliness of the vessel into which the beer is poured, the tendency of insoluble solids or regions of localized high surface area to cause gas bubbles to form, and most critically, surface active and other components of beer that either promote and sustain foams or tend to destroy them. Such factors have been reviewed recently (2).

For good foam quality, it is essential that the gas content and dispensing conditions are correct. However, it is just as important that the beer contain sufficient amounts of the materials that promote foam, at the same time being deficient in foam-negative factors. Both types of material are summarized in Table II. Ethanol falls in both categories as it promotes foam at low concentrations (typically up to 4–5%, v/v), but it is detrimental to head when present in greater quantity.

Of the substances listed in Table II, the only positive factors capable of forming a stable foam in their own right are the polypeptides, which can truly be said to form the "skeleton" of a beer foam. Such polypeptides largely arise from proteins in malt and barley or wheat-based adjuncts (wort replacements). They are not provided by sugars or syrups (wort extenders), which

TABLE I
Factors Influencing Beer Foam

Factor	Effect
Gas content	Carbon dioxide
	Nitrogen
Method of dispensing and container	Bubble size
	Gas entrainment
	Detergents
	Grease and contamination
Nucleation	Beer clarity
	Glass damage
Beer composition	Positive and negative factors

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accordingly are diluents of foam-stabilizing materials. Use of the latter can result in beers of inadequate head retention.

The polypeptides of greatest foam-stabilizing character tend to be those that are hydrophobic (8), and as a consequence they tend to be more readily lost from solution. Throughout the conversion of barley to malt and malt to beer, foam-potentiating proteins and polypeptides are lost by hydrolysis and precipitation (Fig. 1). Such losses will be particularly debilitating to foams of beers brewed from a grist of lower nitrogen content.

To circumvent deficiencies of foam polypeptide in such beers, it has been proposed that they be replenished in beer by direct addition of foaming protein after the stages at which the greatest losses occur (4). In particular, the use of egg white (albumen) as a source of such foaming protein was pursued and patents applied for (3). Aspects of the application of this material are now described.

EXPERIMENTAL

Materials

Dried egg white was obtained from Sigma-London Chemical Company, as were ovalbumin, conalbumin, lysozyme, and the various proteolytic enzymes, except for Alcalase, which is a product of Novo.

Production of Egg White Solutions

Dried egg white was suspended at 50 mg/ml in deionized water with a gentle agitation for 30 min. After centrifugation at 20,000 *g*, clarification was completed by filtering through a Whatman No. 1 filter paper.

TABLE II
Beer Components and Foam

Positive Factors	Negative Factors
Polypeptides	
hydrophobicity	Low molecular weight peptides?
size	Lipids
carbohydrate	(Ethanol)
Melanoidins	
Bitter substances	
Metals	
Added stabilizers—e.g., PGA[a]	
(Ethanol)	

[a]PGA = Propylene glycol alginate.

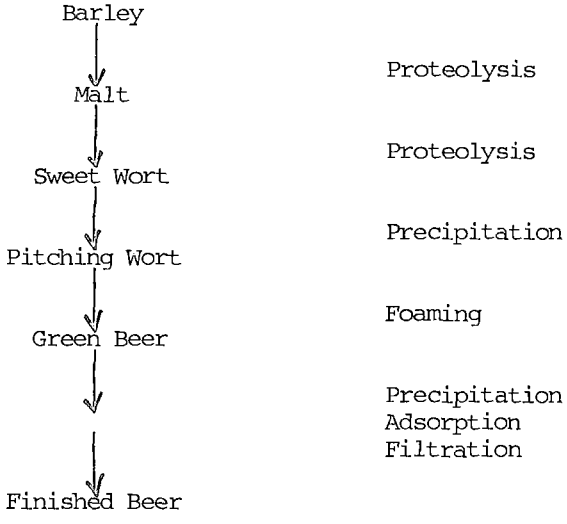

Fig. 1. Potential loss of protein during malting and brewing.

Hydrolysis of Egg White

Solutions of egg white were acidified to pH 1.5 using hydrochloric acid before hydrolysis for 30 min at 50° C with pepsin (0.01 mg per gram of albumen). The mixture was then adjusted to pH 6.5 using sodium hydroxide and heated to 65° C for 1 hr before clarification by centrifugation.

Determination of Foam Stability

Foam stability was determined either by the method of Rudin (7) or by simple shaking in measuring cylinders (4). Lacing index was determined as described by Jackson and Bamforth (5).

For quality appraisal of beer foam, a panel of 6–12 people was simultaneously shown a series of glasses containing control and various test beers, poured using as consistent a procedure as possible. They were asked to judge the head quality on a scale of 1–10, being encouraged to judge the beers within an experiment over as full a range of the scale as possible.

Other Measurements

Haze was measured by Radiometer hazemeter (Radiometer-America, Inc., Westlake, OH) using formazin standards. In breakdown trials, haze was determined after cycles of 24 hr each at 37.5 and 0° C.

Ultrafiltration of Beers

Beers were filtered through a Millipore Pellicon cassette system using a membrane of nominal molecular weight limit of 1,000.

RESULTS AND DISCUSSION

Egg white has been shown to be superior to other bulk protein sources with respect to its head rentention properties at low concentrations (4). An example of the extent of improvement to beer head which is possible from the addition of 100 ppm protein (from a solution of spray-dried whole egg white) is shown in Figure 2. Thus, whole egg white can be used to improve the foam performance of beers. Its introduction would typically be to green beer after fermentation.

However, it is well known that egg white is precipitated by relatively mild heating. As expected, the pasteurization of beers containing albumen leads to the development of unacceptable hazes in the product (Fig. 3). Such haze development renders unacceptable the use of untreated egg white for beers that are subjected to heat sterilization, which in practice is the majority worldwide.

The approximate composition of egg white is given in Table III. Three of the proteins in egg white have been confirmed as having good foaming properties in beer (Fig. 4). Lysozyme may act by cross-linking foams produced by other proteins. Our trials

Fig. 2 The improvement in head of a lager beer from the addition of unhydrolyzed albumen. Control beer, **left,** beer with albumen (0.1 mg/ml), **right.** The beer was of OG 9° P.

revealed that conalbumin and (especially) ovalbumin were qualitatively the most important potential foam-promoting agents in egg white. When added to beer, it was apparent that conalbumin is the protein that imparts the greatest tendency to give hazes upon even the briefest periods of pasteurization (Table IV). Clearly, whereas ovalbumin itself is haze-potentiating, conalbumin is especially troublesome in this regard.

Evidently, means to separate out conalbumin from egg white preparations should benefit use of albumen as a foaming agent in beer. However, the residual ovalbumin would still present a risk to beer clarity. Thus, it was resolved to eliminate from egg white the tendency to cause hazes in beer by alternative, broadly applicable means, while retaining foam-promoting characteristics.

TABLE III
Composition of Egg White

Component	Egg White Solids %
Ovalbumin	54
Conalbumin	13
Ovomucoid	11
Lysozyme	3.5
Ovomucin	1.5
Flavoprotein-apoprotein	0.8
Ovoinhibitor	0.1
Avidin	0.05

TABLE IV
Effect of Heating on Beers Containing 100 ppm Added Protein

	Period of Heating at 65°C (min)	Haze[a]
Ovalbumin	0	1.00
	10	1.40
	20	1.99
	30	2.20
Conalbumin	0	1.18
	10	>12
	20	>12
	30	>12

[a]Measured in EBC formazin haze units as determined according to European Brewing Convention method 7.13.

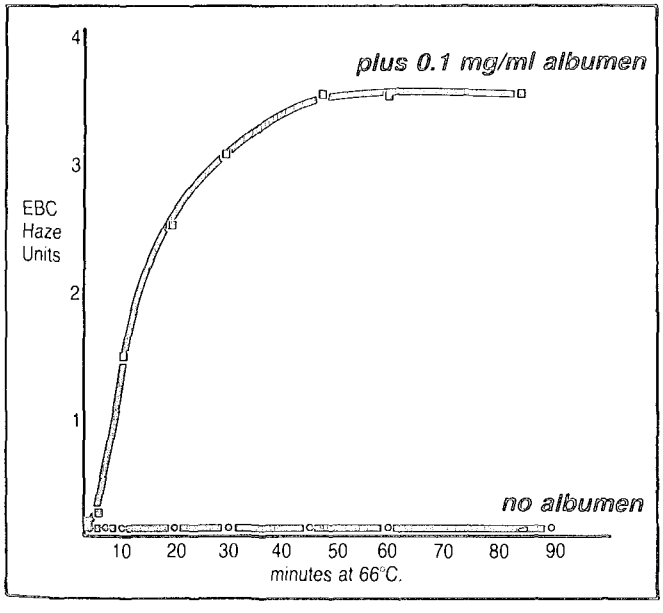

Fig. 3. Effect of tunnel pasteurization on haze of beers containing albumen. The time scale indicates the period elapsing in transfer of beer in glass bottles from 20 to 6° C.

The hydrolysis of egg white by pepsin at pH 1.5 has been shown to remove the haze-forming tendency under rigorous tunnel pasteurization regimes (Table V); treatment for just 30 min at 50° C is sufficient. Other proteolytic enzymes (e.g., Alcalase, bromelain) were tried but with little or no success, possibly because egg white contains materials that inhibit protease action (e.g., ovomucoid and ovoinhibitor, Table III). Proteases with alkaline pH optima were tried with the aim of facilitating regulation of the extent of protein hydrolysis through use of a pH-stat (6). Because of the intransigence of egg white to hydrolysis by such enzymes, it is necessary to control albumen degradation by careful specification

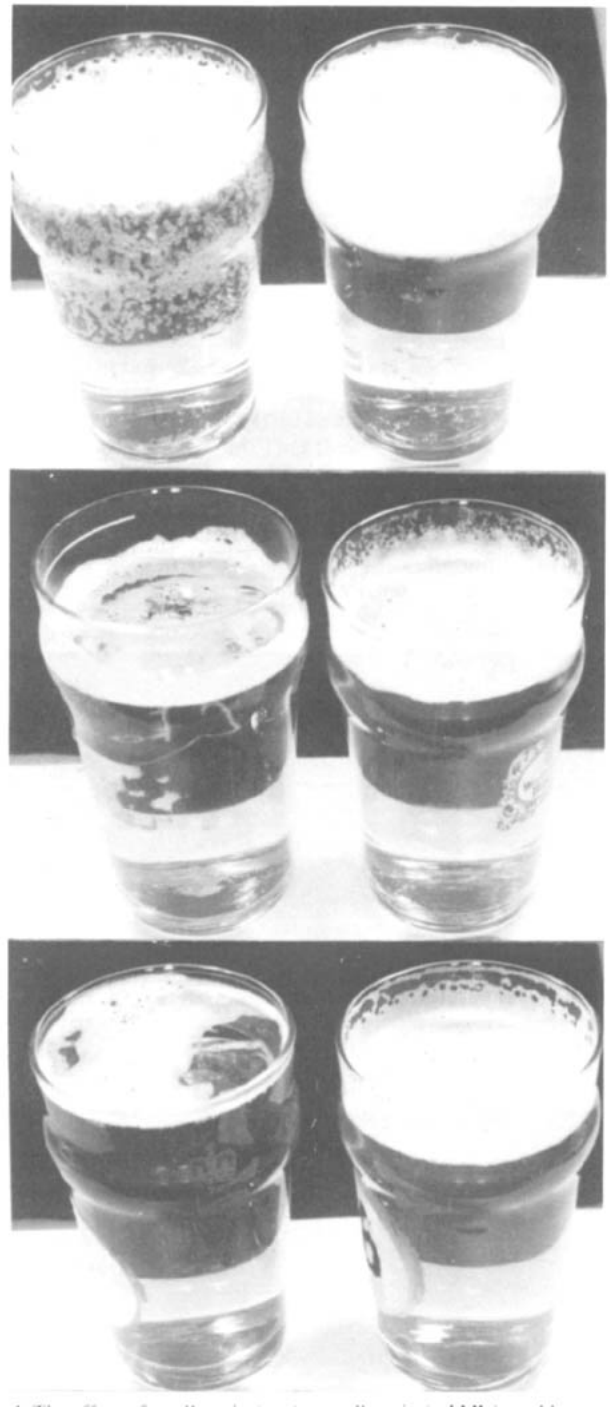

Fig. 4. The effect of ovalbumin **(top)**, conalbumin **(middle)**, and lysozyme **(bottom)** on beer foam. Control beers containing no added protein are shown on the left. Proteins were added at 0.1 mg/ml to a lager beer (OG 7.5° P).

of operating conditions (substrate and pepsin concentrations, pH, temperature, hydrolysis time, and post-hydrolysis enzyme denaturation regime). However, the results of simple shaking trials suggest that periods of hydrolysis of up to 2 hr are not to the major detriment of foam stability (Fig. 5). Furthermore, it is clear that hydrolysis actually *elevates* the tendency of egg white to produce foams (Fig. 6). That hydrolyzed albumen still acts to the benefit of beer foam was demonstrated by adding it in different quantities to a beer and using a panel to qualitatively assess the improvement to head (Fig. 7). This was supported by numerous quantitative observations on many beers, for example the effect on the cling of foams from two beers (Table VI).

The influence of hydrolysis of egg white on its foaming parameters assessed by the method of Rudin (7) is shown in Table VII; hydrolysis lowers by a factor of 10 the K_{prot} of albumen and raises its maximum head retention value (HRV_{max}). As explained elsewhere (1), K_{prot} is a measure of the affinity of a protein for the bubble wall: the lower the value, the less protein is needed to give a stable foam. HRV_{max} is the maximum foam stability, i.e., that occurring at infinite protein concentration. Thus, when assessed in solutions of 4% ethanol, it is evident that the foam potential of hydrolyzed albumen is superior to that of its unhydrolyzed counterpart—less of the hydrolyzed material would be needed to afford a foam, and the resultant foam would be the more stable.

Another commercial whipping/foaming protein preparation, soy, has been compared with egg white (Table VII). Whereas a very stable foam can be produced, it nonetheless demands levels of protein far above those needed from egg white. This would be unacceptable from aspects of cost and colloidal stability.

In practice, there is some dichotomy between the observations made for proteins in such "pure" solution and those in more complex media, such as beer. Thus, we have seen instances of beers whose heads were significantly improved by unhydrolyzed egg white, whereas the hydrolyzed material is of little or no benefit. It is likely that the unhydrolyzed egg white alone can take part in

TABLE V
Pasteurization of Beers Containing Hydrolyzed Albumen[a]

Period of Pre-hydrolysis of Egg White (hr)	Haze After Pasteurization[b]
0	2.33
0.5	0.49
2	0.49
4	0.48
6	0.58
8	0.54
24	0.55
48	0.70
Control, no albumen	0.36

[a] 120 pasteurization units (at 65° C) was afforded to the beers containing 0.18 mg/ml of protein.
[b] Measured in EBC formazin haze units as determined by European Brewing Convention method 7.13.

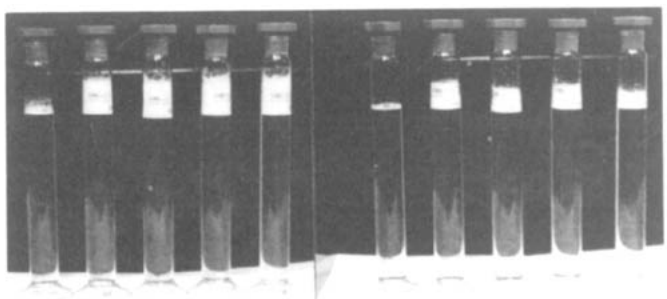

Fig. 5. The effect of hydrolysis on stability of foams produced from egg white. Periods of hydrolysis for cylinders from left to right, are, 0, 30, 60, 90, and 120 min in each photograph, which represent **(left)** standing of the shaken cylinders for 3 min and **(right)** for 60 min.

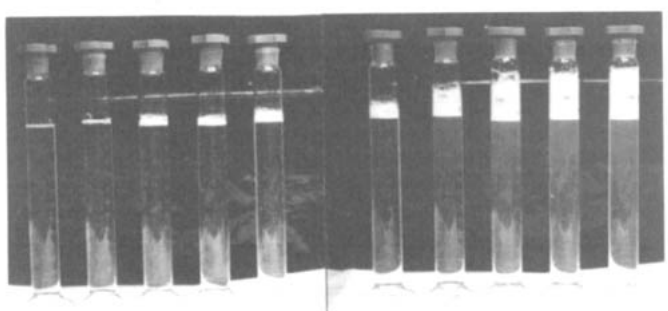

Fig. 6. The effect of hydrolysis on the ability of egg white to produce foam. Successive measuring cylinders contain higher concentrations of protein. **Left,** 0–0.2 mg/ml unhydrolyzed; **right,** 0–0.2 mg/ml hydrolyzed.

TABLE VI
Effect of Hydrolyzed Albumen on Foam Cling

Sample	Lacing Index: Imported Beer	Lacing Index: UK Beer
Control	1.98	4.15
Plus 100 ppm hydrolyzed albumen	2.99	6.26

TABLE VII
Relative Foam Properties of Proteins[a]

Protein	K_{prot}[b] (mg/ml)	HRV_{max}[c] (sec)
Unhydrolyzed albumen	0.78	103
Hydrolyzed albumen	0.074	128
Commercial soya whipping protein	7.7	167

[a] Determined using the Rudin procedure from solutions in 4% (v/v) ethanol.
[b] K_{prot} is a measure of affinity of protein for the bubble wall. Lower values mean less protein is needed to give a stable foam.
[c] HRV_{max} = maximum head retention value.

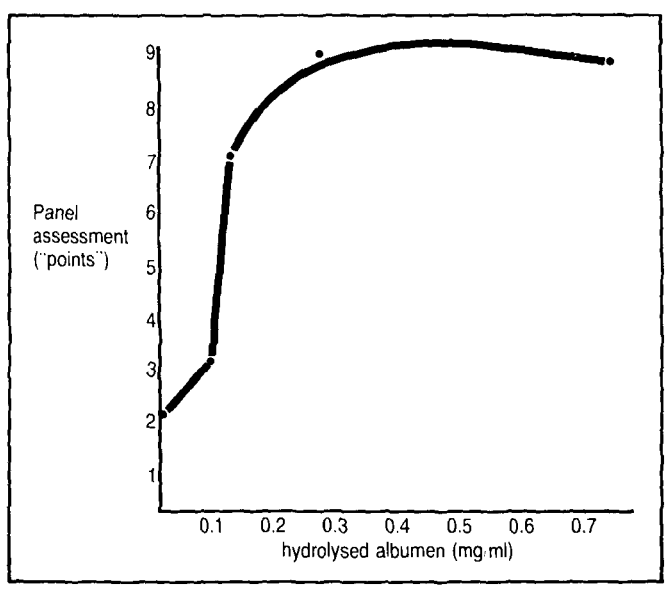

Fig. 7. Effect of hydrolyzed albumen on appearance of beer head. Points indicate the same beer to which different quantities of hydrolyzed egg white were introduced.

interactions with other beer components and render stable a foam in ways over and above being a simple protein backbone. Furthermore, there will be competition between various components in a beer for spaces in the bubble wall, competition that does not occur in, say, a measuring cylinder shake test or Rudin tube. Native egg white may have an additional advantage in a competitive situation in that it can denature at the gas/liquid interface, whereas the hydrolyzed material may be too small to denature and hence readily returns to the bulk beer matrix. The indroduction of factors that will denature, and thus fix, the hydrolyzed albumen in the bubble wall may thus be advantageous.

Hydrolyzed albumen has only a marginal effect on the already excellent foam stability of many of the beers in production in our company. This is because such beers already contain a sufficient quantity of foaming protein. However, beers produced in other parts of the world using high proportions of protein-free adjuncts would be expected to be deficient in foam polypeptides and hence improved by hydrolyzed albumen. The situation is illustrated in Figure 8.

The HRV of beer 1, which was shown to contain 3.2 mg/ml protein, can be increased significantly by the addition of further protein in the form of hydrolyzed albumen (Fig. 8). This is as to be expected, for the native protein of this beer has a K_{prot} of 1.25 mg/ml. In other words, at a protein concentration of 1.25 mg/ml, HRV will be 50% of maximum. Only when the protein content reaches 5–10 times this level (i.e., 7.25–12.5 mg/ml) should its maximum foam potential be realized. As there is only 3.2 mg/ml protein present, addition of further protein in the form of hydrolyzed albumen has a significant effect.

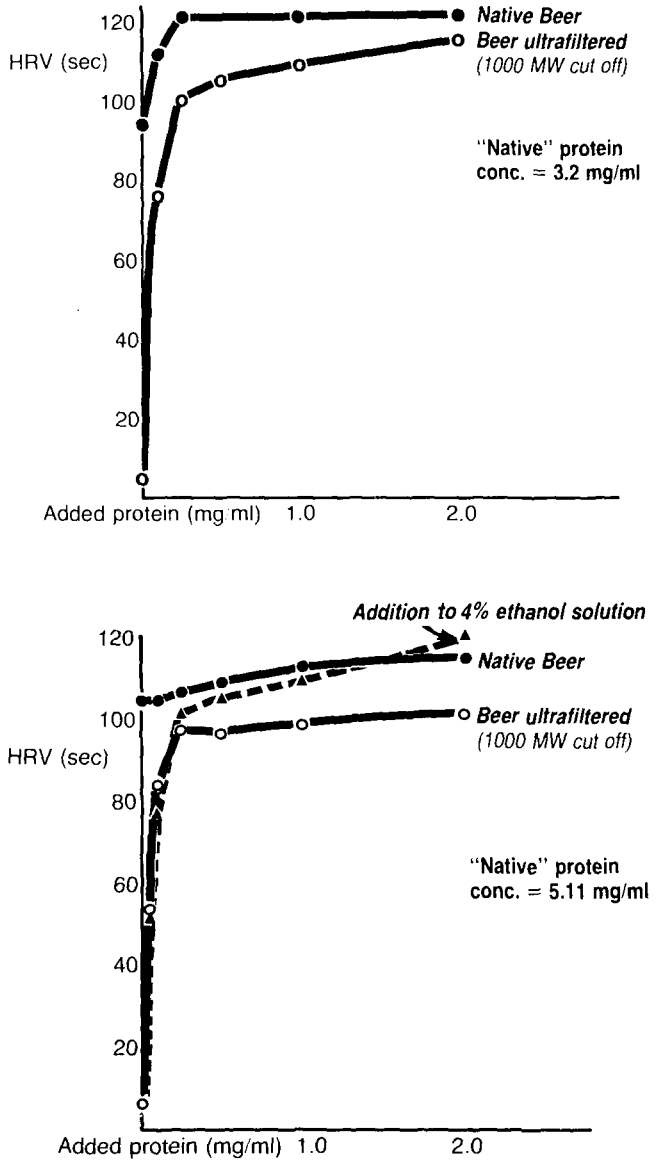

Fig. 8. Addition of hydrolyzed albumen to beer 1 (**top**) and beer 3 (**bottom**).

When beer 1 was ultrafiltered to removed all polypeptides greater than approximately 1,000 mol wt, foam stability of the resultant beer was extremely low. However, the addition of very small amounts of hydrolyzed albumen restored the bulk of the foam potential to the beer. Thus, very low levels of albumen (in this case approximately 0.3 mg/ml) were able to return the HRV of the beer to the value that is afforded by 10-fold more protein of "natural" origin.

Similar results were obtained for another beer (beer 3, Fig. 8), which was, however, of somewhat higher protein content. Thus, the addition of further protein in the form of hydrolyzed albumen was of lesser benefit for this product. Clearly the effect on head from additions of hydrolyzed albumen is a means for assessing the extent to which they are deficient in foam-active proteins. In the case of beer 3, it is apparent that some other deficiency is prevalent. Thus, while it is again demonstrated that low levels of hydrolyzed albumen can restore foam potential to the ultrafiltered beer, the HRV ultimately obtained reached a plateau without achieving the magnitude obtained when hydrolyzed albumen was foamed in 4% ethanol alone. This is most readily interpreted in terms of there being another component(s) in the ultrafiltrate that interferes with foaming. This may be lipid. Thus the comparison of the foaming of hydrolyzed albumen in beer ultrafiltrates with that in a solution of equivalent ethanol concentration may be useful for identifying potential problems with foam-negative materials in beer.

A mathematical model to explain the competition between albumen and native beer polypeptide for places in foam bubble walls is described in the Appendix.

Hydrolyzed albumen does not influence the flavor or aroma of beers and affords no risk to their stability. For example, beer containing this substance shows no greater tendency to colloidal deterioration (Fig. 9). As the addition of hydrolyzed albumen at 100 pm will introduce no more than 0.5 ppm α-amino nitrogen to a beer, it does not present any increased microbiological risk.

The relative merits of hydrolyzed albumen and propylene glycol alginate (PGA) were considered. It is important to recognize thay they act in different ways. Thus, PGA is not in itself a foam forming agent, and unlike albumen it can in no way provide the basic foam matrix that is crucial to satisfactory head performance. PGA rather is a foam protectant, acting to preserve beer head in the face of the destabilizing influence of lipids; hydrolyzed albumen was

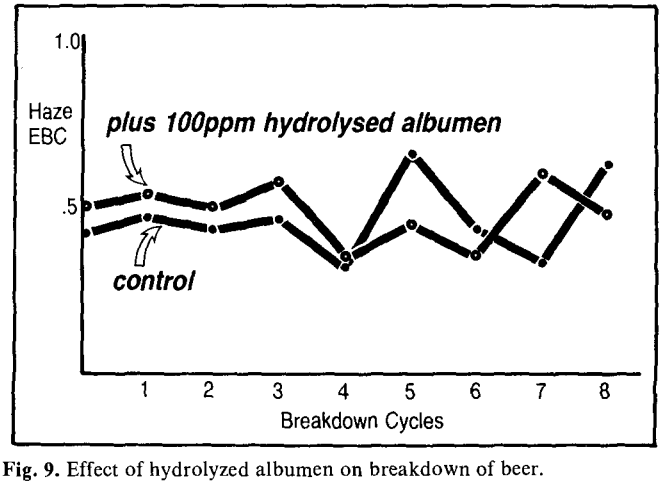

Fig. 9. Effect of hydrolyzed albumen on breakdown of beer.

TABLE VIII
Effect of Hydrolyzed Albumen as a Lipid Protectant

Amount of Albumen (ppm)	HRV[a] (sec)			
	No Lipid	+1 Drop Milk	+2 Drops Milk	+3 Drops Milk
0	79	+[b]	+	+
50	82	+	+	+
100	93	10	5	+

[a] HRV = retention value.
[b] + = insufficient foam produced to enable measurement by Rudin procedure.

found to be of less merit in this regard (Table VIII). There appears to be scope for the use of both materials in the pursuit of excellent all-around head performance.

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APPENDIX

Mathematical Analysis of Foaming

It is proposed that the foaming of polypeptides can be treated as a surface adsorption phenomenon, namely, the saturation of a bubble wall, and as such can be treated according to Langmuir's Adsorption Isotherm and analagously to enzyme reaction kinetics.

Thus, if X is the bubble surface (the area of which will clearly depend on gassing regimes, Rudin sinter characteristics, etc.)

A is egg polypeptide
and
B is beer polypeptide
Then

$$X + A \rightleftharpoons X - A \xrightarrow{k_3} X - A'$$

Then

$$X + B \rightleftharpoons X - B \xrightarrow{k_4} X - B'$$

where X − A and X − B represent the initial state when the respective polypeptides enter the bubble wall and X − A′ and X − B′ represent the form in which they are foam stabilizing, presumably after interaction with other beer components.

Let x = "concentration" of bubble surface
a = concentration of egg protein
b = concentration of beer protein
c = concentration of egg protein adsorbed to bubble (X − A
d = concentration of beer protein adsorbed to bubble (X − B)
K_a = dissociation constant of X − A
K_b = dissociation constant of X − B.

Applying the law of mass action

$$K_a = \frac{(x - c - d)\,a}{c}$$

and

$$K_b = \frac{(x - c - d)\,b}{d}$$

Eliminating d,

$$c = \frac{xaK_b}{K_aK_b + K_ab + K_ba}$$

Alternatively, eliminating c,

$$d = \frac{xbK_a}{K_aK_b + K_ab + K_ba}$$

Let V_3 =rate of conversion of egg white foam to a stable foam
and V_4 =rate of conversion of beer protein foam to a stable foam

$$V_3 = \frac{k_3xaK_b}{K_aK_b + K_ab + K_ba}$$

and

$$V_4 = \frac{k_4xbK_a}{K_aK_b + K_ba + K_ab}$$

When the bubble walls are saturated, then $HRV_{max}^{egg} = k_3x$ ar $HRV_{max}^{beer} = k_4x$, respectively.
Thus

$$V_3 = \frac{HRV_{max}^{egg}aK_b}{K_aK_b + K_ab + K_ba}$$

and

$$V_4 = \frac{HRV_{max}^{beer}bK_a}{K_aK_b + K_ba + K_ba}$$

Taking reciprocals

$$\frac{1}{V_3} = \frac{K_a}{HRV_{max}^{egg}a}\left(1 + \frac{b}{K_b}\right) + \frac{1}{HRV_{max}^{egg}}$$

and

$$\frac{1}{V_4} = \frac{K_b}{HRV_{max}^{beer}b}\left(1 + \frac{a}{K_a}\right) + \frac{1}{HRV_{max}^{beer}}$$

V_3 and V_4 can be equated to observed HRV measureme caused by egg and beer, respectively. Clearly it is assumed that th are not mutually interactive other than by competition for sites the bubble wall.

It is apparent from this treatment how the variations can occ in the extent to which albumen can influence beer foam. T observed head retention will depend on the relative abilities of b and egg polypeptides to enter into the foam (i.e., K_a or I compared with K_{prot}) and then on their relative ability to stabil that foam (k_3, k_4).

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