

A SCIENTIFIC BASIS FOR BEER FOAM FORMATION AND CLING

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Received, 23rd July 1973

The most complex types of nitrogen compound in beer (here termed "proteoses") are, when not combined with polyphenols, highly surface active and hence concentrate in the foam. With unhopped beer, the foam is of the "liquid-viscous" type, which eventually drains and collapses leaving no residual solids. The iso- α -acids in hopped beers are also surface-active, so they too are concentrated in the foam. At the corresponding concentration in bulk solution the solubility limits of some isohumulates* are exceeded. Thus we believe that solids are similarly formed in the bubble films, so that these are reinforced and stiffened to give "cling". One consequence of the increased concentration of iso- α -acids in the foam is that there can be precipitation of iron, nickel, cobalt and zinc isohumulates. This results in concentration of these metals in foam.

The most significant finding is that the concentration of both proteoses and iso- α -acids in the foam exceeds the bulk solubility limits and results in the formation of proteose-isohumulate salts which make the main contribution to the solid reinforcement of bubble films.

These findings make it possible to explain satisfactorily "lacing" or "cling" and also isohumulone losses (a) during boiling and trub separation, (b) during post-fermentation bittering, and (c) during any subsequent foam formation. An explanation is also provided why losses of bitterness and of head retention are linked. Consequently, linked recovery of both bitterness and head retention can occur, as has been found in some recent fermentation improvements, such as continuous fermentation.

Key words: *beer, foam, iso- α -acid, metal, method, protein.*

INTRODUCTION

IN the late thirties of this century it appeared that scientific problems of beer foam had been neatly and conclusively solved. This followed the findings of Ross & Clark,²¹ who had shown that the behaviour of beer foam could be related to the foaming of liquids in general by the use of " Σ "—the average lifetime of a bubble, and also those of Blom,⁷ who had shown that foam decrease followed a logarithmic law. At this stage there appeared to be a satisfactory physico-chemical basis for and a satisfactory method of measurement of the foaming power of beers.

Subsequently to that period, however, a number of people have become dissatisfied with these views and have proposed many other methods of foam measurement. It is

a matter for regret that, in attempts to be strictly "practical," many of these methods, and also studies of head retention in general, have moved steadily away from the scientific to the purely empirical (Cook⁸). It therefore seemed worthwhile to see how far a reassessment could help towards providing a scientific basis for the evaluation of beer foam.

One reason why many people have been dissatisfied with the older views has been that a number of workers have claimed, contrary to the Blom finding, that the decrease of foam with time is not strictly logarithmic. We shall show, in a later paper,⁶ how these divergent views can be reconciled. The chief reason, however, why the older theories on foam are now regarded as unsatisfactory is that they fail to deal with the phenomenon of "lacing" or "cling". This aspect is of paramount practical importance, so, in this paper, the main object

* The term "isohumulates" is used in this paper as an abbreviation for "salts of iso- α -acids."

has been to make "cling" amenable to scientific treatment.

Study of previous physico-chemical papers on beer foam shows that the concept of viscosity has been firmly in the minds of their authors. It follows therefore from this that they have been making the tacit assumption that beer foam is a liquid, whereas our evidence suggests that this supposition is erroneous. Our study of the literature suggests that this error has been made in other fields of enquiry as well.

In dealing with these non-liquid films we shall refer to "solid" or "solid-reinforced" films as an adequate description of their behaviour, rather than considering the fine distinctions drawn by physical chemists between solid condensed, liquid crystalline (mesomorphic) and solid films *sensu stricto*.¹⁰

In seeking an explanation of "cling" we have conducted two main groups of experiments, (a) those in which the nature of the bubble film has been studied chemically and (b) those in which the behaviour of foams and of surface films on beer has been studied physico-chemically. The experimental results obtained are numerous and the experimental details are extensive enough to clog the arguments, so in many cases the arguments have been set out first with references to the relevant experimental matter and results, which are given in subsequent sections.

1. STUDIES ON THE PHYSICAL BEHAVIOUR OF THE BEER BUBBLE FILM.

(a) *Foam Tower Experiments.*—In the primary experiments we isolated foam fractions using a tube inclined at 30° to the vertical since this allows better drainage than a vertical tube. This is similar to the arrangement used by Grey & Stone¹⁵ but a larger quantity of beer (50 litres) and a wider diameter tube (76 mm) were used. In some of the experiments the foam fraction was re-foamed in a smaller tower. The collapsed foam yielded a liquid with solid matter in suspension and in many cases we have analysed the liquid and solid fractions separately. The results are given in the experimental section.

(b) *Surface Rupture Experiments.*—A beer bubble film consists of two opposed beer surface films separated by liquid. One method which we have found very en-

lightening is to study the single film on the surface of the beer. A number of lines of evidence suggested that bubble films and also surface films on hopped beer are solid or solid-reinforced. If this is so, then the surface film can be visualized as an exceedingly thin skin of ice, with any solid object frozen in the surface. In turn this makes obvious the unsuitability of using a surface viscometer for measuring the "viscosity" of the hopped beer surface film, since the viscometer uses the damping of successive oscillations of a disc immersed in the surface layer, when it is obvious that any solid film will be repeatedly broken and no true measure of its strength will be obtained.

In consequence we invented a measuring device consisting of a simple flat metal disc which is immersed in the surface of beer and supported in the centre by a highly elastic filament, either a long, very thin steel wire or a human or horse hair. This is supported at the top by a clock mechanism which can rotate at a constant speed of one rpm. It will be clear that the rotation builds up a torque which is transmitted to the disc. This can rotate freely in a liquid film but is "frozen" in a solid one until there is sufficient torque to break the surface film, so the time to this occurrence is measured.

When this instrument was used with a hair support to measure the surface resistance of water, the disc started to revolve after 7–8 sec. With unhopped beer the time was 12–18 sec; after the addition of 8 ppm of pure α -acids the disc started to revolve after 45 sec, while after the addition of 16 ppm of iso- α -acids rupture only occurred after 6 min 50 sec. After the addition of 30 ppm of iso- α -acids, rupture had not occurred after 8 min so the drive was stopped and the apparatus left for 1 hour. After this, the liquid was lowered from the disc, which then started to revolve until all the stored torque had been released. These tests show that the instrument is able to demonstrate and measure the degree of "solidity" of the surface film on beer. At the same time they strongly support the view that the surface film on unhopped beer is a liquid one, whereas there is a solid or solid-reinforced film on hopped beer. This is a key concept, so that we have devoted considerable time to obtaining related evidence and to exploring the consequences which follow.

(c) *Phenomenological Observations.*—A number of simple observations have a bearing on the problem. A liquid-film foam on an unhopped beer should drain completely to give a clean glass, while the solid, or solid-reinforced, films of hopped beer foam should remain on the walls to give "lacing" or "cling." That this occurs has been shown by Klopper¹⁸ and confirmed by ourselves (Experimental Section I).

A striking observation of the same type can be made at the end of head retention tests in the Rudin apparatus. When the drainage tap is opened at the end of the test, the foam from unhopped beer runs out quickly and completely. On the other hand, the foam from hopped beer remains in the tube and washing with large volumes of water is needed to remove it.

Another simple observation is the "day after" test. If a liquid foam such as that on unhopped beer is exposed to drying conditions, then, given sufficient time, the liquid between the bubble walls will drain away, and the films will collapse into the drained liquid and will cease to exist. On the other hand, solid-reinforced films under the same conditions will dry as the liquid drains to give a persistent film. This film or foam residue is, of course, frequently seen.

A further illustration is provided by some beers with good head retention properties. With these it is possible, with a suitable pen, to write one's initials on the surface film and the markings will persist for some hours. We consider that this would be impossible if the film were liquid.

The same phenomenon can be illustrated in a different way by measuring the rate of fall of small glass spheres through a head of foam. We have made such measurements on foam on unhopped beer and have obtained reasonably concordant results ranging between 0.5 and 4.0 sec and giving an average of 0.8 sec. However, after addition of 25 ppm of iso- α -acids the behaviour was quite different. Then, if after foaming the sphere is placed very gently on the surface, so that this is not ruptured, the sphere can remain on the surface for periods up to 22 sec. This placement was very difficult to achieve successfully, so that extremely variable times were recorded.

These observations help to establish the liquid nature of unhopped beer foam and

the solid, or semi-solid, nature of hopped beer foam. It was therefore desirable to obtain chemical evidence on this aspect and on the nature of the substances taking part.

2. STUDIES ON THE CHEMICAL NATURE OF THE BEER BUBBLE FILM

Various substances in beer might conceivably play a key part in causing the phenomenon of "cling," so the various possibilities were studied in turn.

(a) *Proteose Participation in Foam.*—There have been a number of papers showing that the complex nitrogenous substances of beer are concentrated in beer foam (Gray & Stone¹⁶; Mischke¹⁹; Klopper^{17,18}; Gjertsen¹⁴) while our own work (Experimental Section VII) agrees with these findings. For reasons given elsewhere (Bishop^{2,4}) these complex nitrogenous substances are considered to be below true protein in molecular size. In consequence the term "proteose" is used here for the substances involved in foam formation, without exact definition of molecular size beyond implying that they are below true protein and above peptides.

Proteoses are concentrated in foam because of their surface activity and they therefore must play a part in head retention. Further evidence of their importance is given by the reduction in head retention caused by the action of proteolytic enzymes (Trolle²⁴; Posada *et al.*²⁰) or caused by precipitation with tannic acid (Klopper¹⁹). These tests also show that it is only the complex part of the nitrogen compounds which plays a part. Our model experiments, given later, also demonstrate the essential part played by proteoses in foam formation (Experimental Section III).

The evidence of Bateson & Leach¹ shows that it is the more basic proteoses which take part in foam formation; while the very convincing and direct immuno-electrophoretic evidence of Grabar & Daussant¹⁴ shows that the complex nitrogenous constituents of bubble films are present in roughly the same proportions as they are in beer or in beer haze. This was found true both of films formed by Gjertsen¹³ with CO₂ and by Grabar & Daussant with air. They identified a derivat from one of the barley albumins and a smaller proportion of another probably derived from hordein.

Our own results, given later, agree in general with their findings but some differences in detail from haze amino-acid composition may be significant.

(b) *Participation of Polyphenols in Foam.*—One of our early hypotheses was that, as in haze, polyphenols might be associated with proteoses and so carried to the head, where oxidation to insoluble films could occur.

However, we have accumulated a considerable body of evidence showing that polyphenols do not play a significant part in bubble film formation. The evidence may be summarized as follows:

- (1) Polyphenols are not concentrated in the liquid part of collapsed foam.¹³
- (2) Our analyses of insoluble bubble film material indicate that polyphenols are not present. (See under (c), (3) below).
- (3) Addition of polyphenols to beer tends to reduce head retention (*e.g.* addition of tannic acid, Klopper¹⁸). In our tests (Experimental Section IV) addition of hop polyphenols had no significant effect on head retention.
- (4) In a later paper⁶ it will be shown that closely similar foam collapse times are obtained if air or nitrogen is used for foaming the beer. This shows that oxidation does not play a part in strengthening bubble films and is consistent with the non-involvement of polyphenols.

It was therefore concluded that the constituents of bubble film must be sought elsewhere.

(c) *Iso- α -acids.*—The evidence given earlier shows that solid bubble films and "lacing" are definitely associated with hops, while the evidence from the previous paragraph shows that the polyphenols of hops are not responsible. There is on the other hand a strong body of evidence that iso- α -acids play an important role in head formation. This evidence may be summarized as follows:—

- (1) Bitter substances, identified in later papers as iso- α -acids, have been shown to be concentrated in foam and diminished in the remaining beer (Grey & Stone¹⁵; Mischke¹⁹; Klopper¹⁸; Gjertsen¹³ and our own results, Experimental Section II).

(2) Addition of iso- α -acids to beer is known to increase head retention and induce "lacing" (Whitcar & Button;²⁵ our own results Experimental Section I)

(3) In our own tests on solid bubble film material from a foam tower concentrate, thin layer chromatography and ferric chloride staining were used after acid and also after alkaline hydrolysis.⁹ The acid hydrolysate showed the absence of polyphenols. The alkaline hydrolysate gave a single spot in a different position from the five spots given by the polyphenols of haze material, but corresponding to the main spot given by similar treatment of iso- α -acids. The alkaline hydrolysate showed a bathochromic shift in ultraviolet light, indicating a polyhydroxylic substance (Experimental Section V).

(4) Our model experiments also showed that iso- α -acids, in conjunction with proteoses, strongly promote head retention and are essential for lacing (Experimental Section III).

At each stage in the development of our ideas we have checked our findings with beer by means of model solutions. For these the base was a 5% alcohol-water solution and proteoses were added as Oxoid "Peptone P," which contains more complex bodies than most other bacteriological peptones. The iso- α -acids, as in our other tests, were carefully prepared from very pure crystalline mixed α -acids.

In a typical test, a set of nine beer glasses was arranged as a Latin square each with 200 ml of 5% alcohol containing 0, 100 or 300 ppm of proteoses and 0, 30 or 90 ppm of iso- α -acids. The results, after gassing with nitrogen under standard conditions, indicate that proteoses alone do not form a persistent head, while iso- α -acids alone give a moderate head with no lacing. On the other hand, the two together give a very persistent head with strong lacing. This, together with other evidence, points to these two as the main constituents of beer foam.

While neither proteoses nor iso- α -acids separately formed a persistent head, there was nevertheless evidence that each separately was surface-active and so concentrated in the head. Surface activity implies that the molecule has both hydrophobic and

hydrophilic groups, so that it orients itself in the liquid surface with the hydrophobic group projecting outwards. With iso- α -acids it seems likely that the iso-pentenyl side chain is the hydrophobic group.

(d) *The Precipitation of Metallic Isohumulates.*—Addition of trace amounts of metals such as iron, nickel and cobalt to beer is known to improve head retention but it is worthwhile noting that this amelioration only occurs in the presence of iso- α -acids.^{12,23} We were able to show that addition of a few ppm of nickel greatly increased the film strength on the surface of beer (Experimental Section VI). As a result we were led to consider how this could happen.

Obviously at tolerable iso- α -acid concentrations in beer, nickel and such metals do not form bulk precipitates or they would have been removed from clear beer, but the key conception came from the realization that, because of their surface activity, iso- α -acids are present in bubble films in much greater concentrations, and therefore at these concentrations, insolubilization of iso- α -acids could occur.

The evidence as to the actual concentrations of iso- α -acids likely to be present in the head came from our own work and from the careful work of Gjertsen¹³ for the E.B.C. Haze and Foam group. In three tests, he foamed 50 litres of lager with CO₂ in a foam tower and obtained around 2 litres of collapsed foam liquid. The beer started with 25–26 ppm of iso- α -acids which in the foam liquid had risen to 93–120 ppm. In our work with beers of 25 to 30 ppm iso- α -acids in foam towers we have obtained similar concentration of iso- α -acids (Experimental Section II).

It seemed likely that the concentration of iso- α -acids found in the collapsed foam gave a good indication of the concentrations existing in the bubble walls. It followed that, at these concentrations, it was possible that precipitations could occur which did not occur in the main bulk of the liquid.

The next stage therefore was to investigate the precipitation of metal isohumulates in iso- α -acid solutions of the order of concentration found in foam. For these tests, equivalent amounts of metals as acetates were added in 25% excess to a 5% ethanol solution, containing 95 EBU, which was adjusted to pH 4. The liquids were then

centrifuged and the clear liquids acidified and extracted as in the normal estimation of iso- α -acids. The results were not precise but gave the following indications of the order of solubility (expressed as EBU).

Potassium	90
Magnesium	92
Zinc	77
Cobalt	84
Nickel	64
Iron	59
Copper	38

It follows that, as might be deduced, these metals would not be precipitated in the beer itself, but that the last five metals would be precipitated in the greater iso- α -acid concentration existing in the head. In consequence, concentration of these metals in the head would be expected, and in the present tests we found that this occurred. We also found that trace metals tended to be removed from the body of the beer fairly early in the foaming process (Experimental Section II, Table VI). The picture presented by the combined results was that, either when the initial concentration of trace metals was very low or when some had already migrated into the head, the remainder stayed in the bulk beer. This was presumably because the solubility product of the respective isohumulates was then not exceeded. When concentrations were such that the solubility products were exceeded then the trace metal isohumulates would be expected to be precipitated in the foam. In this connection we made an interesting observation during our solubility tests given above. It was noticed in these, especially with magnesium and zinc, that a visible surface film of isohumulate was formed on the liquid and this film persisted even after 30 min centrifuging at 18,000 rpm. The most probable explanation of this accumulation is that the molecules are highly oriented with respect to their hydrophobic and hydrophilic groups.

It is well known that the elements iron, nickel and cobalt have a very strong influence on head retention, and, corresponding to this, we found a low solubility indication for their isohumulates. There is, however, with these and with copper the complication that oxidation can occur to increase the insolubilization of the film. This

may explain irregularities in the apparent solubility figures.

The high solubility of the sodium isohumulates suggested that these might tend to reduce the concentration of iso- α -acids in the head. Tests appeared to confirm this, since increase of sodium content reduced the head retention figure for a beer (*e.g.* addition of 1000 ppm of sodium as the chloride lowered the head retention from 108 sec to 102 sec). However, the addition of sodium ions to unhopped beer also led to a similar reduction in head retention, so the cause appears to lie elsewhere.

While unexplained, the phenomenon has been identified and in consequence the effects of the sodium and potassium concentrations in beer need consideration as a possible contributory cause of poor head retention.

(e) *Precipitation of Other Basic Substances.*—The findings with metal isohumulates suggested the possibility that other basic substances present in beer might be precipitated in the head by iso- α -acids. Consequently, ammonia and a number of aliphatic amines were tested. The results showed that these were not significantly precipitated by 150 ppm of iso- α -acids.

(f) *The Concept of Proteose-Iso- α -acid Interaction.*—The observation of the greatly improved head when both proteoses and iso- α -acids are present gave rise to the suggestion that, in a manner analogous to acidic polyphenols, the acidic iso- α -acids could combine with the basic proteoses and so improve head, just as they do by combining with some metals.

In the first tests of this, a portion of Sephadex G 10 was added to a solution containing proteoses and iso- α -acids. This should absorb only the simpler molecules, including water, and should thus concentrate in the unabsorbed solution all molecules with a molecular weight in excess of 700. Measurement of iso- α -acids before and after showed that these had not been concentrated, which they should have been had they combined irreversibly with any proteoses. This was confirmed in another type of test, as it was found as easy to extract iso- α -acids quantitatively (after acidification) from proteose solutions as it was from plain water-alcohol solutions. (This was apart from a slight delay probably caused by

adsorption of proteoses at the liquid interface.)

At first sight these experiments appeared to negate the idea of proteose-iso- α -acid interaction, but evidence continued to accumulate in favour of the idea. The next stage was to conclude that, as with metallic isohumulates, conditions in the head might be different from those in bulk because of the higher concentration there of both iso- α -acids and proteoses. The simple test, therefore, was to increase the concentration of iso- α -acids in the main bulk of the beer to that likely to occur in the head. The result was immediate and considerable precipitation in all beers tested, including an (originally) unhopped one (Experimental Section I, Table I). One commercial beer, where the head retention appears to be due mainly to iso- α -acids, showed much less precipitation than the others; it is known that the proteose content of this beer is low.

This work suggested that iso- α -acids could act as acidic protein precipitants comparable with well-known ones, such as trichloroacetic, salicyl-sulphonic and phosphotungstic acids. Direct tests showed that iso- α -acids precipitated not only basic proteins such as clupein and salmine but also more neutral proteins such as egg albumin. It is evident from the tests with Sephadex that the compounds precipitated from beer are not complexes but freely dissociable salts. This must be so, otherwise the foam films formed, *e.g.* in filling bottles, would not redissolve and the beer would contain bubble ghosts.

While precipitation of metals by iso- α -acids has previously been studied, we believe it is a new concept that iso- α -acids can act as precipitating acids to proteins and protein-related substances. Further, we believe that this concept leads to a number of fruitful deductions, which are developed partly below and partly in a later paper.

(g) *The Composition of Proteose-Iso- α -acid Precipitates.*—In the early stages of the investigation the solids from the foam liquid obtained from beer were analysed. They showed a high proportion of complex nitrogenous substances but a low iso- α -acid content. This can now be explained as due to washing the precipitates in methanol during the "purification" procedures. In later treatments this step was omitted and

the iso- α -acid content of direct foam solids approached more nearly those of the precipitates formed by addition of iso- α -acids to beer. From our subsequent knowledge of the dissociable nature of this type of compound, it is now evident that re-precipitation should take place from solutions containing about 100 ppm of iso- α -acids. Given this, the iso- α -acid and proteose contents of the two types of precipitate would be expected to be similar. In both types the polyphenol contents were low (Experimental Section VII). This suggests that the combination of polyphenols with proteoses results in compounds with low surface activity.

Three of the iso- α -acid precipitates were analysed for amino acid composition. In Experimental Section VII the average amino acid proportions are compared with the average amino acid composition of haze precipitates. The latter averages are those for the analyses by several laboratories of five different haze preparations. These have been summarized by Djurtoft.¹¹

In general the average amino acid analyses of the haze preparations agree with the averages of the analyses of our iso- α -acid precipitates and with the immunological findings¹⁴ that protein fragments from albumins contribute the chief part and derivatives from hordein a lesser part. This is shown in haze preparations and in the iso- α -acid precipitates by rather high proportions of glutamic acid and proline, while lysine is correspondingly low. However some differences between the haze amino acid composition and the isohumulone precipitate composition have been noted. Our results for the latter indicate a higher proportion of aliphatic monamino acids (alanine, valine, leucine and isoleucine) and a lower proportion of aromatic amino acids (tyrosine and phenylalanine) together with a very low content of cystine. It needs extensive tests to see if these differences are meaningful. It will be recalled that it has been shown that haze-forming complex nitrogenous substances are high in sulphur.¹⁶

3. THE PRESENT FINDINGS IN RELATION TO PRACTICE

As a result of the present findings a number of apparently unrelated problems can now be explained and brief indications are given here.

(a) *Iso- α -acid Losses in Boiling and Cooling.*—Starting with an unhopped beer, stepwise addition of iso- α -acids leads to increasing precipitation and it is clear that with hopped wort the corresponding precipitation must have occurred during boiling and cooling and in the fermenting vessel. This, therefore, indicates precipitation by proteoses of iso- α -acids as one, if not the chief, cause of the loss of iso- α -acids which occurs during and subsequent to the normal boiling of hops. In addition, concentration of iso- α -acids must occur in the foam formed during boiling so that another potential source of loss occurs here.

(b) *Gains in Some Fermentation Systems.*—Two relevant phenomena have been observed in continuous fermentations (Bishop³), and, to a lesser extent, in conical fermentations and bottom fermentations. These are a saving in hop bittering substances and, for the same EBU content, an increase in head retention. So far these have appeared to be unrelated and unexplained. However, as the result of the present work, the explanation and relationship seem to be clear. Foam is formed on the beer in fermentation and this foam contains interacting proteoses and iso- α -acids. In batch fermentation these are skimmed off with yeast, whereas in continuous fermentation the foam is stirred in contact with the beer. Consequently, with better contact and with increase in alcohol concentration, part at least of the foam is able to redissolve, with the effects noted. This is comforting in that it means that perfectly normal and desirable constituents are added back to the continuous beer.

(c) *Loss of Iso- α -acids on Post-fermentation Bittering.*—Losses,²⁵ sometimes quite high losses, of iso- α -acids added in post-fermentation bittering have been frequently reported. It is now clear that, if additions are made, even of very pure iso- α -acids, in such a way as to produce high local concentrations, then precipitations will occur similar to those we have deliberately produced by high iso- α -acid additions. Re-solution is a slow process, so that filtration or some other method of clarification at this stage will result in loss. In our view the only entirely satisfactory way of adding post-fermentation bittering is by means of the tangential mixer, so that high local concentrations are avoided.

(d) *Loss of Head Retention in Glass Washing.*—For a number of years quaternary ammonium compounds ("quats") were the best available agents for cleaning and sterilizing glasses in public houses (Bishop *et al.*⁵). The defects found were that the washed glasses had a "greasy" feel and, especially in some beers, there was loss of head retention. These observations were not explained at the time, but the causation is now clear. Addition of the strongly basic quats in an appropriate concentration to beer, or to an iso- α -acid solution, causes precipitation of the quaternary ammonium isohumulate. In beer this reduces the head and, since the precipitate adheres to glass, this acquires a greasy feel.

(e) *Possible Methods of Improving Head Retention and Cling.*—The new viewpoint presented here has suggested a number of possibilities for maintaining and improving head retention in beers. These suggestions are under test at the present time and it is proposed to publish the findings in the near future.

RESULTS AND EXPERIMENTAL

Section I

Iso- α -Acid Additions to Unhopped Beer

A graph of E.B.U. versus head retention from Table I shows that increase of iso- α -acids up to 15 ppm produces striking increases in head retention as measured by the Rudin method.²³ From 15 ppm up to

TABLE I

EFFECT OF ISO- α -ACIDS ON HEAD RETENTION (ADDITIONS TO UNHOPPED BEER)

Iso- α -acids ppm added	E.B.U.* found	Head retention (sec)	Appearance of the beer
0	3.2	80	Bright
10	12.6	98	"
20	21.2	103	"
25	25.5	104.5	"
30	28.7	109.5	Slight precipitate
40	34.8	111	"
50	41.3	114	"
60	48.1	117	"
70	55	121	"
80	61.5	126	Definite precipitate
90	68.1	129	"
100	73.1	131	"
120	82.8	132	"
140	90.8	137	"
160	108.0	138	"
180	112.5	140	"

* Found in solution after centrifuging.

75 ppm there is a steady increase in Rudin values. Above 75 ppm the increase is much slower and is accompanied by precipitation, the significance of which has now been explained.

At the same time, visual observations were made of the effect of the additions on "cling". The unhopped beer gave no "cling", while addition of 10 ppm of pure iso- α -acids gave distinct "cling". This effect was improved up to 20 ppm iso- α -acids, but above this it was difficult to detect noticeable improvement by appearance.

For the measurement of head retention the Rudin²³ method was used, but the results were made more precise by attention to the following points:—

(1) The column was water-jacketed and the temperature maintained at 20.0 ± 0.2 °C, (2) the "holding" gas pressure and the "foaming" gas flow were maintained constant by the use of a manostat and a floatat respectively, and (3) the beers for test were degassed beforehand by centrifuging at 2000 rpm in covered plastic centrifuge tubes, (4) the open end of the foam tube was covered with a loose cap to prevent penetration of air into the foam.

Section II

Foaming Experiments with Unhopped and Hopped Beers

(A) *Unhopped Beers.*—Closely agreeing results were obtained in two experiments: the average results are given in Table II, where it can be seen that there are signs of concentration of metals in the foam even in the absence of iso- α -acids.

TABLE II

FOAMING OF UNHOPPED BEERS

Fraction	Volume (litres)	Apparent E.B.U.	Metals in ppm			
			Mn	Fe	Cu	Zn
Original beer	42	4	0.09	0.18	0.29	0.10
Defoamed fraction	38	4	0.09	0.15	0.30	0.09
Foam	4	4	0.08	0.26	0.44	0.17

(B) *Hopped Beers.*—The first experiment was carried out in connection with the work of the Haze and Foam Group and the average results are given in Table III.

TABLE III
METALS IN HOPPED BEERS OF THE HAZE AND FOAM GROUP EXPERIMENT OF 1969-70
(parts per million of metals in the beers)

Fraction	Na	K	Mg	Ca	Al	Sn	Mn	Fe	Co	Ni	Cu	Zn
Before foaming	197	482	96	24	<0.1	0.04	0.10	0.07	0.04	<0.1	0.27	0.05
Defoamed fraction	197	482	96	24	<0.1	<0.01	0.10	0.02	0.13	<0.1	0.24	0.07
Foam fraction	200	484	100	23	<0.1	<0.01	0.14	0.04	—	<0.1	0.53	0.13

TABLE IV
METALS IN FOAM FRACTIONATED BEER

Fraction	Volume (litres)	Total nitrogen (ppm)	E.B.U.	Poly-phenols	Ca	Mn	Fe	Ni	Cu	Zn
Original beer	45	724	28.5	130	116	0.10	0.10	0	0.29	0.07
Defoamed fraction	42	731	25	125	116	0.10	0.07	0	0.29	0.05
Foam fraction	3	801	69	134	116	0.15	0.32	0	0.46	0.13
Defoamed foam	2	640	10	113	116	0.10	0.08	0	0.21	—
Refoamed foam	1	840	120	130	116	0.21	0.65	0	0.60	—

Among the main elements present, the slight increase in the magnesium in the foam fraction was consistent in the three tests made. Among the trace elements, the levels are all very low and there is little evidence of concentration except with copper, where amounts are higher and there is some concentration in foam.

Subsequent experiments were on a smaller scale but the amounts of trace metals were rather higher and there appeared to be evidence of concentration in the foam of manganese, iron, copper and possibly of zinc, both initially and on the subsequent refoaming.

TABLE V
METALS IN FOAM-FRACTIONATED HIGHLY HOPPED BEER

Fraction	Volume (litres)	E.B.U.	Fe	Cu	Zn
Original beer	4	55	—	—	0.58
Defoamed fraction	3	25	0.33	0.24	0.34
Foam fraction	1	111	0.37	0.45	1.4
Defoamed foam	0.4	106	0.25	0.38	1.3
Refoamed foam	0.6	118	0.32	0.47	1.6

The results for a normally hopped beer are given in Table IV, and for a highly hopped beer in Table V. In further experiments the uptake of metals into the head was followed in successive foam fractions. These results are given in Table VI.

The picture presented by these various experiments is a consistent one. The trace metal isohumulates are freely dissociable, and when present in very low amounts in the original beer (Table III) or in beer after some defoaming (Table VI) they are not significantly removed into the head—presumably because the concentrations together with the iso- α -acids present do not reach the solubility product in the foam. When the trace metals are present in sufficient concentration (Table IV, V; early part of Table VI) they are removed into the head. The picture for iso- α -acids themselves is different: these are surface active and are removed throughout the foaming time (Table VI).

Section III

Model Beer Foam Experiments

Early experiments were made with Oxoid bacteriological peptone. This is low in proteose content and additions of 500 to

TABLE VI

SUCCESSIVE FOAM FRACTIONATIONS OF TRACE METALS AND ISO- α -ACIDS

	Original Beer	ANALYSES OF FOAM FRACTIONS Fractionation Time in minutes							Final defoamed beer
		0-5m	5-15m	15-30m	30-60m	60-90m	90-120m	120-180m	
E.B.U.	29	70	50	60	42	40	39	41	22
Mn	0.10	0.20	0.16	0.22	0.16	0.11	0.11	0.12	0.11
Fe	0.17	0.28	0.22	0.26	0.27	0.23	0.26	0.31	0.28
Cu	0.11	0.46	0.70	0.38	0.16	0.11	0.10	0.10	0.09
Zn	0.04	0.16	0.48	0.05	0.05	—	0.03	0.02	0.02

1500 ppm were required. Later Oxoid Peptone P was used. This is not broken down as far as most "peptones" and additions of 100 to 300 ppm were used.

In the most basic series of experiments, addition of 1000 ppm of Proteose P to water raised the recorded value from 8 sec to 1 min 50 sec. Addition to this of 5% alcohol lowered the value to 50 sec. In a corresponding test, addition of 30 ppm of pure iso- α -acids to water raised the value from 8 sec to 17 sec. Addition to this of 5% alcohol raised the figure to 52 sec.

In a further experiment 100 ppm of Peptone P together with 20 ppm of pure iso- α -acids gave a value of 3 min, showing a strong synergistic effect. Addition of 5% alcohol to this raised the value to 4 min 15 sec, so that the alcohol effect on the iso- α -acids is the dominant one. This appears to indicate some association between the iso- α -acid and alcohol molecules.

Neither proteose alone in 5% alcohol nor iso- α -acids alone in 5% alcohol could be foamed in a foam tower, but with the two together foam could be formed and collected as with beer. This foam could in turn be refoamed. In each case iso- α -acids were concentrated in the foam. The results obtained are given in Table VII.

TABLE VII

FOAMING OF MODEL BEER

(5% alcohol solution containing 77 ppm iso- α -acids and 500 ppm peptone)

	Original "beer" (ml)	Defoamed "beer"	Foam fraction	Defoamed foam	Refoamed foam
Volume	4000	2600	1600	1220	260
E.B.U.	77	58	94	90	94

Section IV

Addition of Hop Polyphenols to Beer

Unhopped beer with a foam half-life of 93 sec was treated with 100 ppm of polyphenols prepared by extraction from hops. This addition had no effect on the half-life and the value was again 93 sec.

A further portion of this unhopped beer was treated with 25 ppm of iso- α -acids and the half-life was raised to 115 sec. With the addition of 100 ppm of hop polyphenols 117 sec half-life was obtained, which agrees within experimental error.

Section V

Hydrolysis of Bubble Film Solids

Acid hydrolysis (Dadic,⁹ Method C) of the solids obtained from foam concentrates showed no spots on thin layer chromatography on silica gel using ultraviolet observation and ferric chloride as stain. The ultraviolet spectrum of the hydrolysate showed no bathochromic shift, indicating the absence of phenols. In the visible region the spectrum showed no maximum at either 450 nm or 550 nm, indicating the absence of anthocyanogens and catechins.

Alkaline hydrolysis (Dadic,⁹ Method A) of the foam concentrate solids, followed by thin-layer chromatography on silica gel, gave with ferric chloride one prominent brownish spot at R_f 0.76. The ultraviolet spectrum showed a bathochromic shift from 318 nm in alkaline solution to 345 nm in acid solution. This is consistent with the presence of a polyhydroxylic compound, which, from the evidence from acid hydrolysis, is not a polyphenol.

Parallel alkaline hydrolysis of beer haze material gave five spots at R_f 0.24, 0.33, 0.40, 0.62 and 0.82: none of these corresponded with the beer foam spot. Parallel hydrolysis of iso- α -acids gave three spots, two at R_f 0.56 and 0.67 and one prominent brownish spot at 0.77, *i.e.* corresponding with that obtained from beer foam material.

Section VI

Addition of Trace Metals to Hopped Beers

It was found that trace metals increased the resistance to rupture of the surface film so much that a wire was used instead of a hair as a support for the disc in our surface film tester. The rupture time for a hopped beer was then 1.5 sec and addition of 1, 2 and 3 ppm of nickel increased the times to 3 sec, 4.5 sec and 8.5 sec respectively.

Section VII

Composition of Proteose-Iso- α -Acid Combinations

Studies were made of the broad general composition of foam solids and also of iso- α -acid precipitates. In the early stages it was not realized that either of these could be dissociable iso- α -acid complexes and consequently preparations were "purified" by dissolving in acetic acid and precipitating by alcohol. In consequence little iso- α -acid was found. Later preparations from foam were made by dissolving in dilute alkali and then neutralizing by hydrochloric acid. The iso- α -acid precipitates were washed in water and re-suspended for analysis.

Analyses showed the ranges in composition given in Table VIII.

TABLE VIII
ANALYSIS OF FOAM CONSTITUENTS

	Foam liquid (Gjertsen ¹⁰)	Foam precipitates (Present authors)	Iso- α -acid precipitates
Ash %	—	—	2.3-3.5%
As % on organic matter			
Total Protein (N \times 6.25)	10.3-10.5%	50-65%	30-44%
Iso- α -acids	1.9-2.4%	2-3.5%	12-16%
Carbohydrates	77-80%	—	—
Polyphenols	0.4-0.7%	1%	4-6%

It is now realized that, because the compounds are dissociable, it would have been better had both the foam and the iso- α -acid precipitates been dissolved for purification and reprecipitation in the presence of 80-100 ppm iso- α -acids.

Polyphenols were low, and in contrast the analysis by parallel procedures of a preparation of haze from stored beer showed 49% "protein" and 23% polyphenols.

The free α -amino nitrogen of the iso- α -acid precipitates was measured and found to be 9 to 15% of the total nitrogen. This confirms the designation of the complex nitrogenous bodies involved as "proteoses", since true proteins would be expected to have around 2% of free α -amino nitrogen.

Amino acid Proportions.—Three samples of iso- α -acid precipitates were hydrolysed and the amino acid composition was analysed by ion-exchange chromatography. The results were reasonably consistent between the three sets, and these are given, with the averages, in Table IX. In this is also given for comparison the average amino acid results by a number of analysts analysing five preparations of beer haze from three breweries. These results are given by Djurtoft¹¹ for the E.B.C. Haze and Foam Group.

The high figures for glutamic acid and proline together with the low figures for lysine indicate that both the haze material and the iso- α -acid precipitates contain some products derived from hordein. Compared with the haze results, glutamic acid and proline are, however, not quite so high in the iso- α -acid precipitate and lysine is rather higher, indicating that a smaller proportion has been derived from hordein. Correspondingly the aliphatic amino acids are higher in the iso- α -acid precipitates. This may help to make these proteoses more surface active because of the "insolubility" of the aliphatic chains. As noted earlier, the proportions of aromatic amino acids (phenylalanine and tyrosine) and of cystine are lower.

Further testing is desirable to see if these findings apply more generally.

Acknowledgements.—We should like to thank most heartily a number of people in the Mortlake Laboratories, including Mr. R. G. Brown and Mr. M. R. Freeston, who have helped in this work.

TABLE IX

AMINO ACID COMPOSITION OF HAZE AND OF ISO- α -ACID PRECIPITATES
(Figures as percentages of total nitrogen as 100%)

Amino acid	Iso- α -acid precipitate				Haze material Average	Iso- α -acid value minus haze value
	(1)	(2)	(3)	Average		
Alanine	5.9	6.0	6.6	6.2	3.4	+
Arginine	8.8	7.7	7.8	8.1	8.5	=
Aspartic Acid	8.2	8.7	9.0	8.6	4.2	+
Cystine + Cysteine	1.0	0	0	0.3	2.5	--
Glutamic Acid	11.8	14.0	14.5	13.4	16.5	-
Glycine	7.6	8.0	8.4	8.0	7.7	=
Histidine	4.5	3.6	3.4	3.8	3.1	=
Isoleucine	3.4	3.1	3.1	3.2	1.9	+
Leucine	6.6	7.1	7.2	7.0	3.37	++
Lysine	5.5	4.0	3.3	4.3	2.7	+
Methionine	0.9	0.6	0.5	0.7	0.6	=
Phenylalanine	2.1	1.7	1.7	1.8	2.4	-
Proline	—	7.6	7.2	7.4	12.2	-
Serine	6.4	5.8	5.8	6.0	4.1	+
Threonine	3.8	3.4	3.6	3.6	2.7	+
Tyrosine	0.9	0.8	0.7	0.8	2.4	--
Valine	5.1	5.5	5.4	5.3	2.8	+
Ammonia	10.5	12.4	11.7	11.5	10.7	-

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