The Effect of Countercurrent Distribution Fractions of Hop Extracts on Beer Foam[1]

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ABSTRACT

This paper covers observations from the first stages of an investigation into the role of the iso-α-acids of hops in the structure of beer foam. Fractions obtained from a countercurrent distribution of water-soluble hop extracts were evaluated for their influence on foam stability. A surprising dual effect (suppressor-enhancer) of isohumulones upon foam was observed. The possibility of some suppressive effect of hop seed lipids upon beer foam is discussed.

Key words: *Fatty acids, Hop Lipids, Isohumulones.*

The "foam tower" apparatus of Gray and Stone (4) was used by our laboratory to concentrate and recover the surface active components which migrate into beer foam. Visual observation of the reservoir and the foam tube during fractionation indicated a systematic sequential change in the type of foam generated as the run proceeded. Beer is known to contain foam enhancers (1). Our observations suggest that there are also substances present whose concentrations could reach a point where they would act as foam depressants, and that the quality of the foam might depend upon a competition between these components at the liquid surface.

The work of Bishop *et al.* (1) very clearly points out that the major foam enhancers have the ability to concentrate and reinforce the bubble film. Foam enhancing properties have often been associated with certain protein or "proteose" fractions, certain metal ions, and hop compounds. The concept of proteose-isohumulone complexes was discussed by Bishop *et al.* (1).

The role of free fatty acids and their possible adverse effect on foam is not fully understood, although their presence in beer has long been recognized and their origin, to some extent, has been investigated. Several low-molecular-weight free fatty acids are known to be by-products of yeast metabolism and are the most abundant in beer. Long-chain fatty acids, mainly in the form of triglycerides, are contributed by the malt and are also known to be present in hops. Most of these lipids are removed during the brewing process and have traditionally not been considered important to beer properties.

Recently, however, more attention has been given to the possible impact of free fatty acids on beer. The analyses of the fractions from our foam tower separations reveal that the fatty acids present in beer also show a tendency to migrate into the foam. The works of Carrington *et al.* (3) and Sandra *et al.* (12) show that certain fatty acids are gushing suppressors at very low concentrations. There is a possibility that this suppressor effect could be the result of these fatty acids competing for sites in the bubble film, which interferes with reinforcement by the proteoses and "isohumulates."

This paper describes certain observations on the unusual effect of isohumulone on foam. The behavior of certain other hop extract components is noted, and the possible contribution of free fatty acids from hop extracts is discussed.

PREPARATION OF SAMPLES

Base Beers

All beers used, with the exception of pilot-brewery-produced, unhopped beer, were commercially finished beers.

Unreduced Isohumulone (Isohumulone)

Prepared from the pure *o*-phenylenediamine complex of humulone (15) by the carbonate isomerization procedure of Howard (7).

Borohydride-Reduced Isohumulone (Rho-Isohumulone)

a) Prepared by the simultaneous isomerization and reduction of pure humulone according to the method of Koch *et al.* (9).

b) Isolated from the center tubes of the countercurrent distribution of various borohydride-reduced hop extracts.

Potassium Salt of Borohydride-Reduced Isohumulone (K-Rho-Isohumulone)

A 35 to 40% aqueous solution of the potassium salt of borohydride-reduced isohumulone; available commercially.

Countercurrent Distribution of Hop Extracts

Apparatus: 100-tube countercurrent fractionator from E. C. Apparatus Co.

Phases: Upper—10 ml isooctane saturated with lower phase.
Lower—10 ml of 0.5 *M* aqueous potassium phosphate buffer (pH 5.5) saturated with upper phase.

Sample: 0.5 g solids introduced on tubes 0 through 4.
Equilibration: 10 min.
Rest: 4 min.
Transfers: 100.
Temperature: Ambient.

On this phase system, both rho-isohumulone and isohumulone have a distribution coefficient of approximately 1; therefore, the same conditions were used for all distributions.

Front Material

Material isolated from tubes 1 through 15 of the countercurrent distribution of various hop extracts. This fraction contains any humulinic acid present in the extract.

End Material

Material isolated from tubes 85 through 100 of the countercurrent distribution of various hop extracts.

Analytical Procedures

a) Iso-compounds were analyzed by dissolving and diluting them with 0.012*N* NaOH in methanol and applying their absorbancy at 255 nm to the following formulas.

$A_{255/52}$ = mg/ml isohumulone in solution read.
$A_{255/47.5}$ = mg/ml rho-isohumulone in solution read.

b) Fatty acids were determined by quantitative gas chromatographic analysis of their methyl esters. The fatty acids were identified and quantified by reinforcement with pure fatty acids from various suppliers. Beer and other liquid samples were extracted and concentrated (with necessary sample size adjustment) by the method of Sandra and Verzele (13).

Gas Chromatography

Instrument: Hewlett-Packard Model 5830A.
Column: 6 ft, 0.25 in. o.d., 2 mm i.d., glass, packed with 10% Silar 5CP on 100/120 Chrom WHP.
Temperature: 150°C for 5 min.
150° to 175°C at 10°C/min.
175°C for 12 min.
175° to 250°C at 5°C/min.
250°C for 15 min.

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Injection: On column at 250°C.
Carrier Gas: Nitrogen at 16 ml/min.
Detector: Flame ionization at 275°C.
Hydrogen 25 ml/min.
Air 300 ml/min.

Foam Decay Analysis

The procedure used is an adaptation of the Blom method (2) as modified by Pierce and Purssel (10). It consists of foaming 200 ml of ultrasonically degassed beer with nitrogen to a volume of 800 ml in a modified 1-liter graduate cylinder. The volume of foam and the volume of the liquid are read at intervals over a period of 50 min. The ratio of the volume of foam to the volume of liquid is calculated, and the log of this ratio is plotted *vs.* time.

The rationale behind this procedure is somewhat empirical: it was noted during the observations of foamed beer that for a short time (8 to 15 min) the foam volume remained relatively constant while liquid volume changed; and after this initial short period of time, the liquid volume remained relatively constant while foam volume changed. This suggested that the liquid volume change was a possible measure of the drainage properties of the foam and the foam volume change a measure of the bubble film stability. Figure 1 illustrates the ability of this procedure to give a visual presentation of both properties on a single display. For the first 10 to 15 min, the value of the ratio of foam volume to foam liquid (VF/VL) is controlled by the change in liquid volume.

In this paper, the emphasis is primarily on foam film strength; hence, the value of VF/VL at 20 min is used to show the effect of various compounds upon film strength. This time is chosen as it seemed to be the time of greatest difference in VF/VL, when the changes measured are due not only to drainage effect but primarily to film strength.

RESULTS AND DISCUSSION

It had been assumed that unhopped beer would have a "poor foam" and that the addition of isohumulone would always result in improvement. Earlier work, however, on another phase of the overall foam study indicated that unhopped beer's foam, while rather coarse in appearance, was surprisingly stable. Additions of small amounts of isohumulone to unhopped beer were found to be a detriment rather than an asset; but when the level of isohumulone exceeded a certain concentration, there was an improvement in foam quality.

Amounts of an aqueous solution of the potassium salt of borohydride-reduced isohumulone (K-rho-isohumulone) necessary to give 0, 5, 10, 15, 20, etc. mg/l. of reduced isohumulone were added to unhopped pilot-finished beer, and the foam decay data were determined at each concentration. Figure 2 shows how small incremental additions of iso-compounds affect foam quality as measured by the foam decay curves. The suppressant-enhancement effect is illustrated by a plot of VF/VL at 20 min *vs.* mg/l. of rho-isohumulone added (see Fig. 3).

In the initial experiments in this series, aqueous solutions of K-rho-isohumulones contained 35 to 40% solids with purities of 85 to 87% based upon rho-isohumulone in the organic solids.

The K-rho-isohumulone was subjected to countercurrent distribution, and three fractions were isolated from selected tubes at the end of the 100 transfers. Figure 4 shows a plot of absorbance at 255 nm of an alkaline methanol solution of the upper phase only

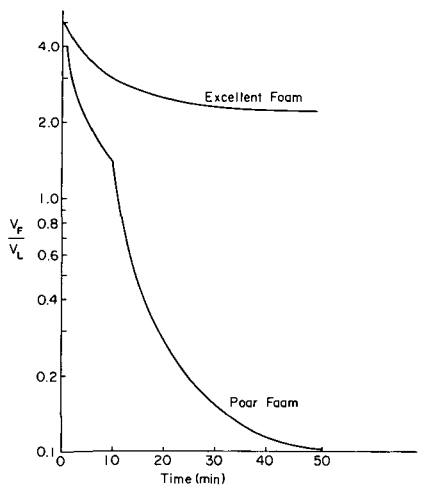

Fig. 1. Example of a "good" and a "poor" foam as could be derived from the "Foam Decay" procedure.

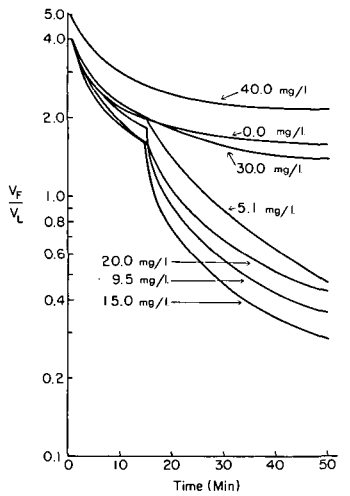

Fig. 2. Effect of various concentrations of added rho-isohumulone on unhopped beer foam.

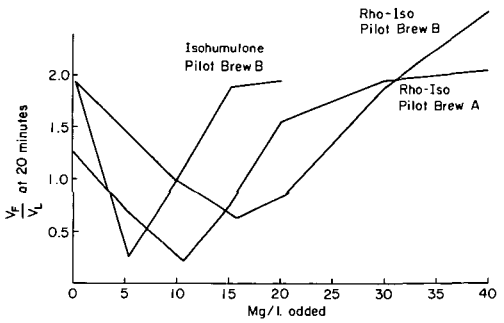

Fig. 3. Effect of various concentrations of iso-α-acids on the foam of two different unhopped beers.

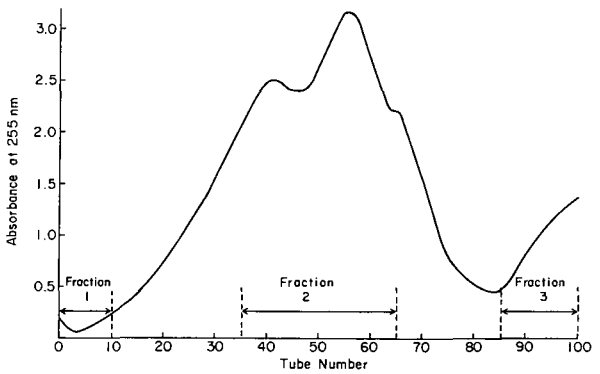

Fig. 4. Countercurrent distribution of K-rho-isohumulone. See text for conditions.

as a function of tube number, and indicates which tubes contained the three fractions collected. The fractions were isolated by removing the isooctane phase, acidifying the aqueous phase, and exhaustively extracting it with isooctane. The combined isooctane was taken to dryness at 60°C under vacuum.

Each of these fractions was added to unhopped beer at concentrations of 0, 5, 10, and 20 mg/l. Figure 5 shows the effect of each fraction on foam stability. It can be seen that fraction 1 (tubes 0–15) had little if any effect on the foam; fraction 3 (tubes 85–100) was increasingly detrimental throughout the entire range of concentrations. Fraction 2 (tubes 35–60) exhibits the same effect

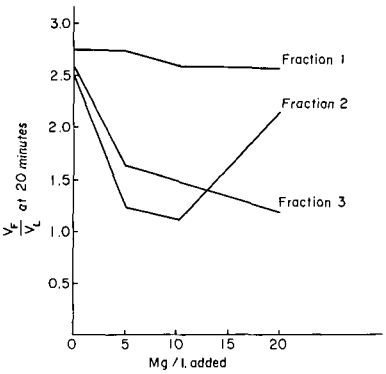

Fig. 5. Effect of countercurrent distribution fractions upon the foam of an unhopped beer.

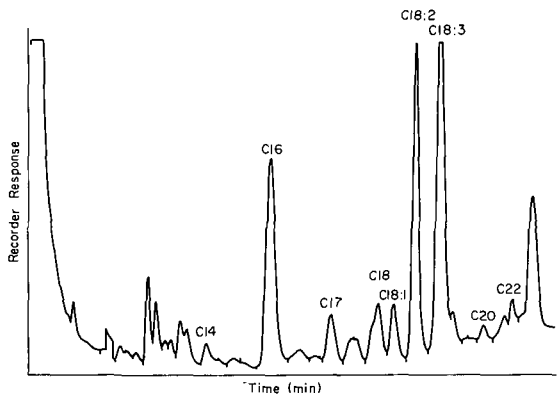

Fig. 6. A typical chromatogram of the esterified end material fraction (tubes 85 through 100) from the countercurrent distribution of a hop extract.

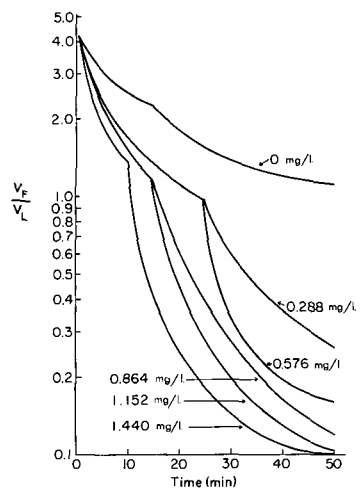

Fig. 7. Effect of pure fatty acids in the ratios found in the hop extract end material fraction upon the foam of a finished beer (the mg/l. values are total fatty acids).

on foam stability as was achieved by the use of the whole K-rho-isohumulone (see Fig. 3). Rho-isohumulone produced by the method of Koch *et al.* (9) also exhibits the same effect. This indicates that the phenomenon is not the result of by-products of the preparation of the potassium salt but rather a true property of the rho-isohumulone.

When this series was repeated with isohumulone prepared by the methods of Wollemer (15) and Howard (7), the same depressant-enhancement effect on foam stability was found, but the concentration needed for maximum depressant effect was less than that found when rho-isohumulone was used (see Fig. 3). This indicates that the effect is a common property of both reduced and nonreduced isohumulone.

The same K-rho-isohumulone which gave the previous results was added to a different pilot-finished unhopped beer. Figure 3 shows that the expected effect upon the foam stability was observed, but again the concentration necessary to give maximum detrimental effect was slightly different.

A possible explanation for the differences observed in concentrations necessary to give maximum depressant effect between these experiments is discussed in the conclusions.

Because the effect of the end material fraction (fraction 3, tubes 85 through 100) on the unhopped beer was suggestive of the presence of a foam depressant, this material was examined in greater detail. When the crude end material fraction was subjected to methyl esterification with boron trifluoride-methanol and examined by gas chromatography, the retention times of certain of the observed peaks were the same as the saturated and unsaturated methyl esters of C12 through C22 fatty acids. Linoleic and linolenic acids were predominant. The end material fractions from several types of pre-isomerized reduced hop extracts were examined in this manner, and all were found to exhibit these same peaks. Figure 6 shows a typical chromatogram of the esterified end material fraction from hop extracts. The end material fraction from all of the hop extracts had a detrimental effect upon the foam of finished beers.

TABLE I
Fatty Acids in Hop Fractions as Per Cent of Total

	End Material Fraction	Hop Seed Analysis	
	From isomerized hop extract	From bullion hops	Literature values (11)
Palmitic	11.5	5.4	7.0
Stearic	4.1	3.4	3.0
Oleic	14.3	7.0	10.0
Linoleic	36.1	65.6	60.0
Linolenic	33.8	18.2	20.0

TABLE II
Analysis of End Material from Various Hop Extracts

	Isomerized Hop Extracts		K-Rho-Iso Hop Extracts		Purified[c] K-Rho-Iso Hop Extract
	A	B	A	B	
Purity[a]	37.4	38.0	85.0	86.1	95.2
% End material as received	36.9	38.3	14.3	12.9	1.5
% Total fatty acid end material	5.6	5.3	7.1	6.9	4.5
C16:0[b]	9.6	10.0	13.4	19.2	11.0
C18:0[b]	6.2	6.1	6.9	5.5	5.7
C18:1[b]	7.8	7.8	6.9	8.8	17.4
C18:2[b]	40.4	40.4	22.7	21.5	26.5
C18:3[b]	36.0	35.7	50.1	45.0	39.4

[a]Purity as % rho-isohumulone in solids.
[b]Fatty acids as % of total fatty acids.
[c]Prepared by the method of Westerman *et al.* (14).

A K-rho-isohumulone extract was analyzed and found to be 85% pure and to contain a 14% end material fraction which contained 7% total fatty acids. If such a material were used as a post-fermentation additive to achieve an additional 10 mg/l. isohumulone, it is possible that approximately 0.3 mg/l. of total fatty acids (C16 through C18) would be added to the beer. An ethanolic mixture of pure fatty acids in the ratio found in the K-rho-isohumulone end material fraction was added to finished beer at approximately this level of total fatty acids. In all cases, there was detrimental effect upon the foam film strength as shown by a 28 to 45% lowering of the value of VF/VL at 20 min. Figure 7 illustrates this depressant effect for one of the beers.

All of the extracts available to us were produced from seeded Bullion hops. Seeds were physically separated from a weighed portion of a sample of these hops. The seeds were ground, extracted with chloroform, and the extract was saponified and analyzed for fatty acids. It was found that the hops contained 23.4% w/w seeds, and 40.7% of the seeds was chloroform-extractable, of which 60.1% of the saponified chloroform extract was C16 through C18 fatty acids. This indicates that approximately 5% of the original hops was C16 through C18 fatty acids.

Table I shows the fatty acid analysis of the hop seeds and that of the end material fraction of a hop extract. The hop seed analysis is in agreement with literature values (11) and resembles the analysis of the end material fraction of a hop extract.

Table II illustrates the results obtained from the examination of the end material fraction from the countercurrent distribution of various hop extracts. It should be noted that the fatty acid pattern remains similar to that of the hop seeds and that the per cent total fatty acids in the end material fraction is roughly the same, when based upon solids distributed, regardless of source. However, the amount of end material fraction found varies greatly from the crude extract to the highly purified K-rho-isohumulone. This means the purer the extract, the less detrimental end material fraction will enter the beer per unit of iso-acid added.

CONCLUSIONS

The suppressor-enhancer effect of isohumulones when added to unhopped beer is at the present time unexplained; however, preliminary evidence demonstrates that it is truly a function of the isohumulone. The difference in concentration necessary to achieve maximum depressant effect between isohumulone and rho-isohumulone could be explained by a difference in hydrophobic properties. The difference noted between the two pilot beers could be due to a variation in the base substrate of the beer itself, as indicated by the difference in the values of VF/VL at 0 mg/l. additions (Fig. 3). It should be pointed out that all observations relative to this effect were made using unhopped beer brewed from a single brewing formula; other unhopped beer was not available.

Various researchers have concluded that the addition of hop lipids to a brew has relatively little effect upon the foam quality of the final beer (5,6,8). These data, however, are usually based upon kettle hopping.

The conditions present in the kettle are not conducive to the saponification of the hop lipids to release the free fatty acids. Kavanagh *et al.* (8) state that less than 0.5% saponification occurs in a 2-hr boil in water at wort pH. The majority of any lipids extracted by the wort are removed with the trub, or during fermentation. However, when hops are ground, extracted with an organic solvent, isomerized with alkali, and processed into a pre-isomerized hop extract to be used as a postfermentation additive, saponification has occurred and most of the previous protection has been lost.

The effect of fatty acids at the levels which could occur by split hopping of beer using pre-isomerized hop extracts has been shown. It is realized that yeast itself contributes these same fatty acids to beer. The effect of μg/l. levels of these fatty acids, in addition to those normally present, would suggest that these possible foam detriments be kept at as low a level as possible. As pointed out by Bishop *et al.* (1), the concentration of substances at the constantly renewing surface of the beer is one of the prime factors in foam film strength. Because the foam depressants also concentrate at the surface, their initial concentration should be as low as possible.

It should not be concluded from the information obtained from this preliminary study that the so-called end material fraction of hop extracts and the fatty acids contained therein are entirely responsible for "poor foam." These findings only indicate that the properties of the above materials may offset the foam-strengthening attributes of isohumulates, proteoses, and certain metal ions.

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