MECHANISM OF BEER FOAM STABILIZATION BY PROPYLENE GLYCOL ALGINATE

By G. Jackson, R. T. Roberts* and T. Wainwright
(*Brewing Research Foundation, Nutfield, Redhill, Surrey, Great Britain*)

Received 27 June 1979

Propylene glycol alginates give greater increases in foam stability than are given by equal amounts of neutral polysaccharides. This is due to electrostatic interaction between carboxyl groups on the glycol alginate molecules and amino groups on the peptides in the bubble wall. This interaction within the bubble wall is responsible for the stabilizing action of propylene glycol alginate against the harmful effects of lipid-like materials in beer foam.

Key words: *beer, foam, alginate, protein, lipid.*

Introduction

The foam stabilizer most commonly used in the brewing industry is propylene glycol alginate (PGA). Although this material increases the stability of foam and improves foam appearance, little is known about how it acts.

It is well known that polypeptides are responsible for the formation of beer foam.[6] These polypeptides have a wide range of iso-electric points, although basic peptides participate preferentially.[13] Other materials, acting in several different ways, exert a considerable influence on the stability of the polypeptide foam. Surface-active materials such as lipids in beer can enter the bubble wall and influence the stability of the foam by causing a discontinuity of the polypeptide film. Lipids in beer cause poor quality foam having larger bubbles. The major function of charged polysaccharides as foam stabilizers is to overcome the detrimental effects of lipid. Roberts *et al*[11] showed that the lipid in beer can exist in two states, termed 'dispersed' and 'non-dispersed' which can be distinguished by observing the shape of the curve obtained by successively diluting the beer and plotting the head retention value at each dilution against the dilution. Lipids in the non-dispersed state are particularly effective in destroying foam on beer but, since all the lipids eventually become dispersed, non-dispersed lipids are only likely to be present in beer if introduced at, or near, the time it is served.

Other materials in beer, such as hop constituents[3] and melanoidins[7] which are not themselves surface-active can contribute to foam stability. Asano and Hashimoto[1] demonstrated that isohumulone can interact with the polypeptides of beer during foaming to give enhanced foaming. They suggested that isohumulone-peptide complexes result from interaction of isohumulone with the amino groups of beer polypeptides. A similar interaction between melanoidins and beer polypeptides has been suggested[7] to account for the foam stabilizing effect of melanoidins.

Large uncharged polysaccharides which are neither surface active nor able to interact with proteins increase foam stability by increasing the viscosity of the liquid draining from the foam.[9] In this paper the effects of PGA on beer foam are compared with those of a neutral polysaccharide and a mechanism is proposed to explain the action of PGA.

Experimental

The PGA used in this work was Manucol Ester B (Alginate Industries Ltd). All-malt unhopped beer, 1040 OG, was prepared in the pilot brewery at BRF.

Head retention values (HRV) were measured by the Rudin method,[12] using carbon dioxide as the foaming gas, except where noted otherwise. Beers were degassed under vacuum overnight and all other solutions were degassed just before addition to the beer. In experiments with diluted beer the required amount of PGA (as 1% w/v solution) or Dextran T2000 (1% w/v solution) was added to 20 ml of the degassed beer and 3·5 ml of ethanol was added. The beer was then diluted with distilled water to 100 ml. The diluted beers were equilibrated at 20°C before measurement of foam stability.

Viscosities were measured in an Ostwald viscometer at 20°C using 3 ml samples of diluted beer prepared as described above. The remainder of the diluted beer was used for measurement of HRV.

Surface viscosities were measured using the method described by Gardner.[5] Beers were degassed by standing in plastic bags overnight at room temperature and were further degassed for 3 h under vacuum. The beer was diluted 1:4 with 3·5% (v/v) ethanol. Half of the beer sample was poured into the measuring dish, taking care to avoid bubble formation, the sample of PGA was introduced, and the rest of the beer was then added. The rate of damping of the disc in the surface was measured at various intervals. Values of surface viscosity were calculated according to the equation given by Bulas and Kumins.[2]

For the preparation of a 'foam-active' polypeptide fraction from beer, samples of unhopped beer were dialysed exhaustively against distilled water and the non-dialysable substances were fractionated on a column of Carboxymethyl Sephadex. The sample was applied in citrate-phosphate buffer, pH 4·0, and, after washing at pH 4·0 to remove non-adsorbed material, the adsorbed fraction was eluted at pH 10·0, dialysed and freeze-dried. Acetylated proteins were prepared from this material according to the method of Fraenkel-Conrat.[4] The foam-active peptide fraction and its acetylated derivative were dissolved in 3·5% ethanol to give concentrations equal to those of the foam active peptide in the original beer and the pH was adjusted to 4·0.

The effect of PGA against lipid in beer was examined by measuring the HRV of the beer at successive dilutions. Hopped beer or the same beer containing 80 ppm PGA was diluted with 3·5% ethanol to give concentrations of beer of 20, 40, 60, 80 and 100%. Linoleic acid was added just before measurement of the HRV (at concentrations of 0·2, 0·4, 0·6, 0·8, and 1·0 ppm respectively) and the beers were foamed with nitrogen, in place of carbon dioxide.

Foam tower experiments were carried out using an apparatus similar to that described by Gray and Stone.[6] The foam emerging from the top of the tower was passed into receivers containing 1% solution of linoleic acid to collapse the foam. Fractions of 30 ml of collapsed foam were collected until foaming ceased and the collapsed foam fractions and the residual liquid were analysed for isohumulone by the Recommended Method of the Institute of Brewing and for PGA by the method of Raible and Engolhardt.[10]

Results and Discussion

The principal surface-active agents in beer are polypeptides which occur with a wide range of sizes and charges. This range is due to the breakdown of the native cereal proteins during malting, mashing and wort boiling. It is of great advantage, when looking for interactions that stabilize foam, to have all the surface-active material (*i.e.* polypeptide) present in the foam rather than only a fraction of the polypeptide which has been selected as a result of the particular method of foaming. In the Rudin apparatus, the same

* Present address: The British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey.

volume of foam is produced for every sample examined so that, as beer is diluted, surface-active material will also be diluted and a point reached when there is just sufficient surface-active material present to form the required volume of foam. For a beer OG 1040 brewed from an all-malt grist, this occurs at about 20% beer, 80% diluent.[11] Most of the head retention measurements described in this paper were made at this concentration of beer.

Most of the experiments described here were carried out using unhopped beers. As mentioned earlier, isohumulone and related compounds derived from hops can stabilize foam by interacting with surface-active material present in the bubble wall. The use of unhopped beers avoids complications arising from the presence of these materials. The effects of any other potentially interfering substances in unhopped beers will have been reduced by using diluted beers since their concentration will have been reduced to only one-fifth of that in undiluted beer.

In Fig. 1 the effects of various concentrations of PGA on the stability of foams from unhopped beer diluted 1:4 are

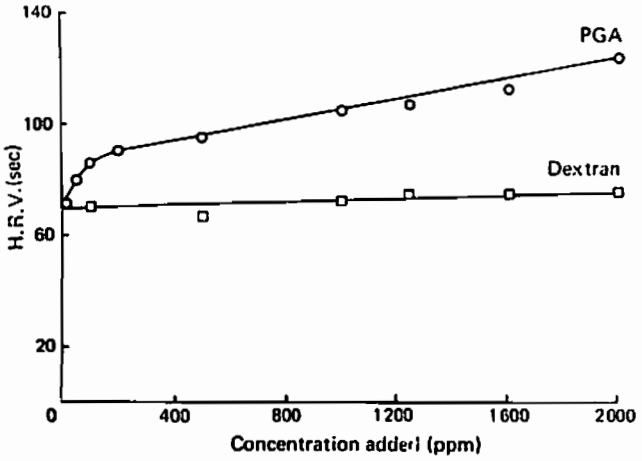

Fig. 1. Relation of head retention value to PGA concentration in diluted beer.

compared with the effects of additions of neutral Dextran T2000 (molecular weight 2,000,000) to diluted beer. There is a difference in the shapes of the graphs of HRV against concentration for additions of neutral and of charged polysaccharides to beer. This has been observed for a variety of polysaccharides. Charged polysaccharides give an initial rapid rise in foam stability followed by a slower rate of increase in HRV with increasing concentration. Thus PGA gives a more stable foam than would be expected from the action of a neutral polysaccharide.

Neutral polysaccharides are known[9] to increase foam stability by increasing the viscosity of the liquid draining from the foam. Charged polysaccharides also increase viscosity and they are known to bind water tenaciously so that even dilute solutions are very viscous. This has led to the suggestion on several occasions that charged polysaccharides exert their effect on foam stability by increasing the viscosity of the beer and thus slowing down the rate of drainage from the foam.

Figure 2 shows the HRV of diluted beers containing PGA or neutral polysaccharide (Dextran T2000) as a function of the viscosity. It can be seen that at low concentrations of PGA there is considerable improvement in HRV with no apparent change in viscosity of the beer, so that at these concentrations it is not improving foam stability solely by increasing the bulk viscosity. At its usual concentration in beer PGA causes only a slight increase in viscosity. At concentrations of PGA which are very much higher than those used in the brewery, viscosity effects may become important.

Klopper pointed out that there is a close correlation between surface viscosity, which measures the stiffness of the

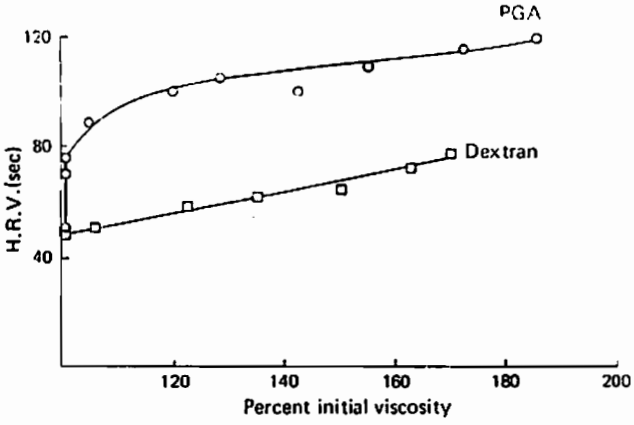

Fig. 2. Relation of head retention value to viscosity.

surface, and foam stability.[8] The effects of PGA on the surface viscosity of a diluted beer are shown in Fig. 3. The surface viscosity of a degassed beer alters only slowly with time, whereas when PGA is present the rate of ageing at the

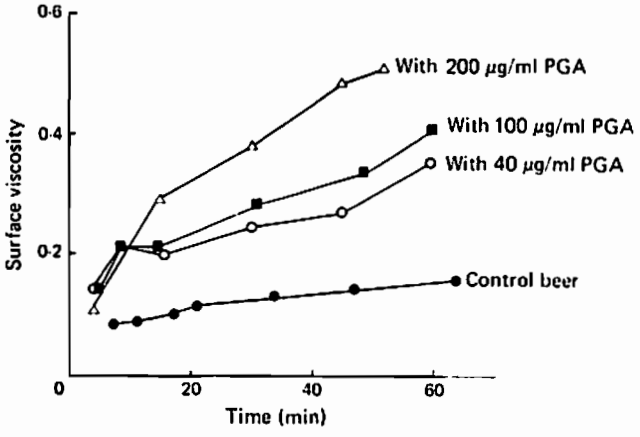

Fig. 3. Change of surface viscosity with time.

surface increases. The higher the concentration of PGA the greater is the rate of ageing. It has been shown[5] that isohumulone also increases the surface viscosity, but its effect is much greater than that with PGA.

In this method of measurement of surface viscosity, the effects of changes in bulk viscosity on surface viscosity measurements are allowed for by comparing the rate of damping of the disc at the surface of the liquid with the rate in the bulk liquid. The increase in surface viscosity caused by PGA might therefore be attributed to interaction with polypeptides at the gas-liquid interface of beer.

A reasonable interpretation for the shape of the curves in Figs. 1 and 2 is that on foaming a beer containing PGA, the charged polysaccharide becomes bound to polypeptide at the newly formed gas-liquid interface and stabilizes the bubble wall. As the concentration of PGA in solution is increased, the amount bound to the wall increases, giving a rapid increase in head retention with increasing concentration of PGA. At high concentrations of PGA, there will be a further contribution to foam stability due to the increase in the viscosity of the liquid draining from the foam.

The nature of the interaction between PGA and beer polypeptide was investigated in various ways. Figure 4 shows the effects of increasing concentrations of sodium chloride on foam stabilization by PGA (80 ppm) in diluted beer. While the presence of high salt concentrations has little effect on the foam stability of the control beer, the foam stabilizing action of PGA is reduced at high salt concentrations. This suggests that PGA is bound to polypeptides in the bubble wall by

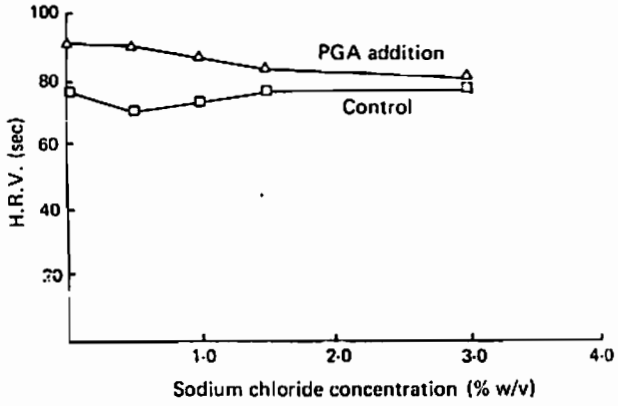

Fig. 4. Effect of salt concentration on head retention value with and without 80 ppm PGA.

electrostatic bonds. Increasing the ionic strength of the liquid around the bubbles causes competition between PGA and ions in solution for the available binding sites on the polypeptide. At high ionic strength the extent of binding of PGA is lessened and the head retention is decreased. The effect of pH on the foam-stabilizing action of PGA in a beer diluted 1:4 with 3·5% ethanol is shown in Fig. 5. PGA causes an

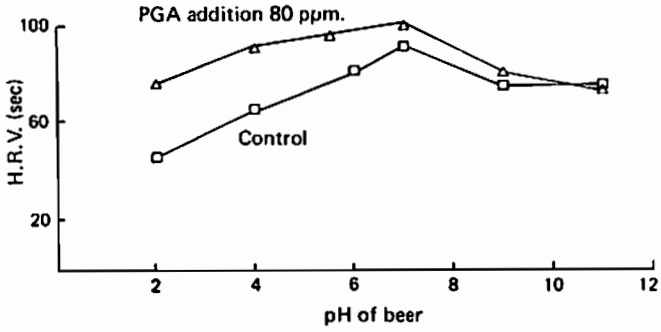

Fig. 5. pH dependence of stabilizing action of PGA.

increase in foam stability at acid and neutral pH, but its foam stabilizing ability is reduced at alkaline pH. This type of behaviour is consistent with the results from the previous experiment and suggests that the interaction is between the carboxyl groups on the PGA with groups such as the free ε-amino groups of lysine, in peptides in the bubble wall. If the charges on these groups in the peptides are neutralized there will be no ionic interaction.

To obtain further support for this hypothesis the effect of suppressing the dissociation of amino groups in beer peptides was examined. A foam-active polypeptide fraction was isolated as described in the Experimental Section. Table I

TABLE I. Interaction of 80 ppm PGA with Beer Peptides before and after Acetylation.

Sample	HRV (sec)
Non-acetylated	47·5
Non-acetylated + PGA	75·5
Acetylated	56·5
Acetylated + PGA	56·0

The foam-active basic polypeptide was isolated from beer and a portion was acetylated (as described in Methods). The acetylated and non-acetylated peptides were dissolved in 3·5% ethanol to give a concentration equivalent to that of the basic polypeptide in the original beer.

shows that addition of 80 ppm PGA to the acetylated 'foam-active-peptide' fraction gives no increase in foam stability, whilst addition of PGA to the same peptide fraction which has not been acetylated gives an increase of 59% in HRV. Acetylation prevents interaction between PGA and amino groups of the peptide so that no stable complex can be formed.

PGA protects beer foam against harmful lipid. This is illustrated in Fig. 6, which shows the effect with linoleic acid

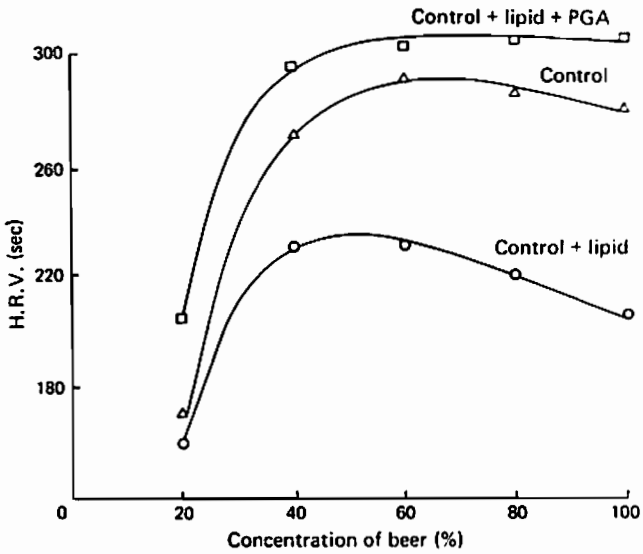

Fig. 6. Efficacy of 80 ppm PGA in combating the effects of linoleic acid (in the non-dispersed state). The concentration of linoleic acid is 1 ppm in 100% beer.

which has been added immediately before the beer was foamed, *i.e.* lipid in the non-dispersed state. As was pointed out by Roberts, the dilution curve can be used as a measure of the quality of the foam on beer.[11] The dilution curve of the control beer is typical of beers with good head retention and having good quality foam. The curve obtained for beer plus linoleic acid is typical of beers with bad foam, and the foam had a coarse appearance, with large bubbles. PGA improved the foam stability of the beer in the presence of lipid and also improved the shape of the dilution curve. Addition of PGA to beers containing lipids usually results in foams having a creamy texture, with small bubble size, and an appearance similar to that of beers not containing lipid. Similar results were obtained using beers to which palmitic acid had been added. Palmitic acid is another of the fatty acids which occur in beer.

Simply increasing the viscosity, using a neutral polysaccharide such as Dextran T2000 in place of PGA, did not improve the stability of foam on beers containing lipid. Furthermore, the dextran did not improve the foam appearance.

Experiments with the acetylated and non-acetylated peptide fraction from beer showed (Table II) that, if interaction

TABLE II. Protective Action of 80 ppm PGA against 2·5 ppm Linoleic acid with Acetylated and Non-Acetylated Beer Peptides.

Sample	HRV (sec)
Non-acetylated peptide	52·2
Non-acetylated peptide + lipid	39·7
Non-acetylated peptide + lipid + PGA	54·9
Acetylated peptide	58·3
Acetylated peptide + lipid	37·5
Acetylated peptide + lipid + PGA	37·5

between peptide and PGA is prevented the protective action of PGA is lost. The lipid was added just before measurement of the HRV so that it was in the non-dispersed state. Addition of PGA to the non-acetylated peptides plus lipid gave foam

of a creamy appearance while foams formed from acetylated peptides containing lipid had a poor appearance even in the presence of PGA.

It seems likely therefore that the effect of PGA is due solely to the interaction with the peptide in the wall, rather than interaction with the lipid. Possibly PGA cross-links peptides in the bubble wall and this maintains the stability of the film even when lipid molecules are interspersed among the peptides.

The amount of PGA which can be bound to the wall, and hence the foam-stabilizing effect of PGA should depend on the number of free basic groups on the polypeptide in the wall, and on the distribution of such groups on the surface of the bubble. These groups are likely to react with the carboxyl groups of alginic acid and such reaction should decrease the number of groups available to react with PGA. Beers were treated with insoluble alginic acid which had been washed three times with water to remove soluble fragments before addition to the beer. After standing overnight at 4°C, the precipitate was removed by centrifugation and linoleic acid and PGA were added before the beers were foamed. As shown in Table III, removal of basic peptides

TABLE III. Efficacy of 80 ppm PGA against 1 ppm Linoleic Acid in Alginic Acid Treated Beers.

Sample	HRV (sec)
(a) Control Beer	
Beer	95·0
(b) Beer treated with 100 ppm Alginic acid	
Beer	97·0
Beer + 1 ppm linoleic	89·8
Beer + 1 ppm linoleic + 80 ppm PGA	95·5
(c) Beer treated with 200 ppm Alginic acid	
Beer	96·5
Beer + 1 ppm linoleic	89·0
Beer + 1 ppm linoleic + 80 ppm PGA	90·2

results in a loss of stabilizing action of PGA against lipid. It is interesting to note that, in this example, removal of the basic peptides caused only a moderate decrease in foam stability of the beer, and there was little change in the appearance of the foam.

It has been shown[1] that isohumulone binds to amino groups of the bubble wall in foam and that it is concentrated in collapsed foam.[6] In a hopped beer containing PGA, both isohumulone and PGA should be concentrated in the foam. This is conveniently demonstrated by using a foam tower. When hopped beers containing 80 ppm PGA were foamed in a foam tower and the collapsed foam analysed, both PGA and isohumulone were concentrated in the foam. Since no quantitative method is available for the determination of PGA it was detected by staining with Victoria Blue and the amount of precipitate estimated visually. The residual defoamed liquid contained only trace amounts of isohumulone and PGA.

The results presented in this paper show that the foam stabilizing action of PGA is due to interaction of the PGA with the bubble walls. The interaction is electrostatic and involves the carboxyl groups on the glycol alginate and amino groups on the peptides in the bubble wall. This binding of PGA to the wall also serves to protect the foam against the harmful effects of lipid. Similar binding mechanisms have been proposed to account for the actions of isohumulone[1] and melanoidins[7] on beer foam.

Like PGA, melanoidins protect the foam against lipids. So do certain other charged polysaccharides such as pectin and carboxymethyl cellulose. Isohumulones have smaller molecules than these substances and their structures are less suitable for cross-linking polypeptides or regions within a polypeptide. This may explain why isohumulones do not protect foam from the harmful effects of lipids.

Acknowledgements.—The authors thank Miss C. M. Grundy for skilful technical assistance.

REFERENCES

1. Asano, K. & Hashimoto, N., *Report of the Research Laboratories of Kirin Brewery Co. Ltd*, 1976, **19**, 9.
2. Bulas, R. & Kumins, C. A., *Journal of Colloid Science*, 1958, **13**, 429.
3. Cook, A. H., *Proceedings of the European Brewery Convention Congress, Estoril*, 1971, 469.
4. Fraenkel-Conrat, H., *Methods in Enzymology, XXV*, Academic Press, 1972, 495.
5. Gardner, R. J., *Journal of the Institute of Brewing*, 1972, **78**, 391.
6. Gray, P. P. & Stone, I., *Wallerstein Laboratories Communications*, 1940, **3**, 159.
7. Jackson, G. & Wainwright, T., *Proceedings of the American Society of Brewing Chemists*, 1978, **36**(4), 192.
8. Klopper, W. J., *Journal of the Institute of Brewing*, 1954, **60**, 217.
9. Krauss, G., *Brewers Digest*, May 1970, 66.
10. Raible, K. & Engolhardt, I., *Brauwissenschaft*, 1965, **18**(10), 398.
11. Roberts, R. T., Keeney, P. J. & Wainwright, T., *Journal of the Institute of Brewing*, 1978, **84**, 9.
12. Rudin, A. D., *Journal of the Institute of Brewing*, 1957, **63**, 506.
13. Wenn, R. V., *Journal of the Institute of Brewing*, 1972, **78**, 404.