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Studies on the physical and compositional changes in collapsing beer foam

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Abstract

Batches of foam were characterised by analysis of conductivity and quantitative changes in mass and solute composition of material retained both within foam and foam drainage. The conductivity of dispensed beer foam declined exponentially during the initial stages of foam collapse, followed by a secondary or consolidation stage characterised by deviation from exponential decay and increases in the concentration of polypeptide material in both foam and foam drainage. Analysis of the amino acid composition of whole collapsed foams suggested that polypeptides of enhanced hydrophobicity were selectively partitioned to the gas–liquid interface at foam formation and subsequent consolidation. Estimates of the changes in total mass of the liquid phase within dispensed beer foam suggested that foam collapse proceeded by two distinct stages of exponential decay characterised by different rates (k_1, k_2). The first rate (k_1) corresponded to the initial stage of foam collapse and accounted for 85–90% loss of foam mass. This was followed by a slower rate of collapse (k_2) which corresponded to the consolidation stage. The results from physical and compositional analyses suggest that the initial stage of foam collapse is dominated by gravitational drainage from a liquid rich foam followed by a change in emphasis to coalescence and bubble rupture during the consolidation and residual stages of foam collapse. These findings contribute to the understanding and characterisation of foam formation and stability.

Keywords: Beer; Foam; Conductivity; Coalescence

1. Introduction

Foams may be defined as colloidal dispersions of gas within a continuous liquid phase at high gas volume fractions. The generation of a foam results in a large increase in surface area and requires an energy input into the system to overcome the counteracting force of surface tension of the bulk liquid phase. This is achieved by mechanical work in the form of induced turbulence or sparging of the gas phase into the bulk liquid. Foam formation increases the Gibbs free energy of the system. All foams are therefore thermodynamically unstable and the initial energy state is restored by foam collapse. Thermodynamic instability may be partially ameliorated by several physical and compositional factors. In particular, the adsorption of surface active molecules at the gas–liquid interface during foam formation reduces surface tension and confers stability to the newly formed surface. For a given foam system, the properties of the adsorbed molecules can significantly influence foam stability [1].

Protein stabilised foams are of considerable importance within food, beverage and bio-processing industries. The surface activity of molecules such as proteins results from the amphiphilic (hydrophilic and hydrophobic) regions within the molecule. Orientation of hydrophobic domains of proteins towards the gas phase results in reduced mutual surface interaction compared to that experienced by the solvent molecules and this consequently lowers surface tension. Furthermore the viscoelastic nature of the adsorbed protein at the gas–liquid interface and underlying sub-surface layers provides mechanisms to counteract deformation or thinning of individual bubble lamellae, reduces gas

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transfer between bubbles and inhibits bubble coalescence [1].

Beer foam is widely regarded as analogous to a protein stabilised foam system in which the principal foam stabilising material is in the form of polypeptide material derived from the solubilisation and proteolytic degradation of cereal proteins during the malting and brewing processes. The presence and persistence of a layer of foam on dispensed beer together with adhesion of foam material to the vessel wall (lacing or cling) during actual consumption are considered highly desirable attributes in the consumer's expectation and subsequent assessment of beer quality.

The behaviour of well characterised globular proteins at gas–liquid interfaces has been widely investigated with the objective of understanding the foam stabilising activity of proteins. [1] In contrast, the heterogeneity of beer and polypeptide fractions has prevented any detailed analysis of similar behaviour of this material. Analysis and prediction of beer foam characteristics has thus been limited to correlation of foam stability with measurements such as total polypeptide content of the bulk liquid [2] or characteristics of crude polypeptide fractions from bulk liquid or collapsed foam such as molecular mass [2,3] or hydrophobicity [4].

Beer foam is unique when compared to other consensus features of beer quality (clarity, colour, flavour and aroma) in that the foam is generated from the bulk liquid immediately prior to consumption. Furthermore, it is evident from simple observation of batches of dispensed beer foam that foam collapse proceeds in a non-uniform manner. Analysis of foam stability based on bulk liquid parameters does not take into account this dynamic nature of foam systems. A more useful approach to the investigation of the role of surface active solutes and the changes occurring within foams would be time based compositional analyses. However, sampling of foam material presents several problems. Batch sampling from within a foam is impractical since this may yield material not representative of the whole foam, especially during the early stages of foam collapse when stratification of foam structure is readily visible. Furthermore, invasive batch sampling will influence the local characteristics of collapse of the remaining foam. Sampling of whole foam systems at successive time intervals requires the generation of a foam for each sampling point and an assumption that the characteristics of each foam are identical. Alternatively, analysis of material drainage from collapsing foams provides information that indirectly relates to the foam itself.

One alternative to biochemical analysis of solutes in foam derived material is to harness a parameter that may be monitored throughout foam collapse without sacrificial sampling. The conductivity of foams is directly proportional to the solute and liquid content and has been exploited in the analysis of model foam systems [5]. A combination of conductivity measurements of dispensed beer foam in conjunction with compositional analyses of foam and drainage material may thus provide a direct indication of the changes occurring within the foam during collapse.

In previous investigations [6] the foam characteristics of dispensed beer were mimicked by manually pouring bottled or canned beer. Whilst this approach is useful for studying an individual beer foam system it is less amenable to reproducible analyses and comparison of foams because of a lack of control of both the rate of foam formation and foam volume. To counter these problems, beer foam was generated from degassed beer by sparging carbon dioxide through a sintered glass plug (porosity 2) in a custom designed apparatus. This enabled precise control of bubble size and rate of gas discharge into the beer.

2. Materials and methods

Samples of commercial ale and lager (Ale A, 3–5% v/v ethanol, 20–30 BU, Lager B, 5% v/v ethanol, BU) were degassed by standing in open containers for 48 h at 4°C. Test material (400 ml) was equilibrated to 20°C and poured into the glass vessel taking care to avoid foam formation. The column of liquid was supported by the back pressure of carbon dioxide supplied by a regulator valve via the sintered glass plug. Foam (100 ml) was generated by sparging carbon dioxide through a sinter at constant flow rate (180 cm^3/min). Immediately following foam formation, the remaining bulk of the beer was rapidly removed via a side-tap. This arrangement enabled conductivity measurements to be obtained on a standard depth of dispensed foam without interference from the bulk liquid phase and permitted continuous removal of liquid drainage.

The conductivity of dispensed foam was measured using a commercial conductivity meter (Jencon) with a probe fitted with two vertical parallel stainless steel sensors (distance between sensors 0.75 cm). The probe was positioned immediately above the bulk liquid in experiments conducted with beer in situ, otherwise the probe was lowered into the bulk beer so that it was located within the foam following removal of the bulk liquid. Conductivity readings were continuously recorded as a stream of digital data (sampling interval of 2.4 s). Material drainage from dispensed foams was continuously collected throughout the course of foam collapse (0–30 min), via the side tap, in time-based fractions (one minute duration per fraction). Fractions were subsequently analysed for yield (weight), turbidity (absorbance at 600 nm) and total protein content (Coomassie Blue dye-binding assay, based on the method of Bradford [7]).

Individual batches of beer foam were prepared under identical conditions (see above), separated from the beer bulk and whole foams were collected in situ after time intervals of 0, 300, 600 and 900 s post-generation. For each foam, the vessel was sealed with parafilm to prevent evaporation of liquid and the foams were allowed to collapse. High molecular mass material (estimated as $M_r > 5000$) was

prepared from these samples of whole collapsed beer foam by precipitation with cold acetone (−6°C) at a sample: precipitant volume ratio 1 : 3 and the precipitated material was lyophilised in a Speedvac centrifugal vacuum drier. The amino acid composition of this material was determined by acid hydrolysis (24 h in 6 M HCl at 110°C) followed by ion exchange chromatography of the hydrolysate (as described in previous publications [3,8]).

Acetone precipitation of samples destined for amino acid analysis resulted in the complete removal of low molecular mass beer components [3]. This was necessary to eliminate the presence of free amino acids in beer and the potential interference of artefacts arising from the hydrolysis of nucleic acid derivatives [8]. The composition of beer and acetone precipitated beer foam material was also characterised by size exclusion chromatography using columns of Superose 12 and Superdex 75 (HR 10/30 supplied prepacked by the manufacturer, Pharmacia Biotechnology). In previous investigations [6,9,10] these high performance chromatography media were demonstrated to enable the fractionation of both high and low molecular mass solutes in a single chromatographic stage. This methodology was also employed to validate the removal of low molecular mass components in samples destined for amino acid analysis. Prior to column chromatography, samples of acetone precipitated material were resolubilised in 100 mM sodium chloride and were briefly centrifuged in a benchtop microcentrifuge (15 000 rpm) to ensure the removal of residual particulate material.

The volume of beer or acetone precipitated beer material applied to the columns was 200 μl. The column was isocratically eluted at ambient temperature and constant eluent flow rate of 0.25 ml/min with an unbuffered solution of 100 mM sodium chloride. The ultra-violet absorbance of the column eluent was continuously monitored at 280 nm using a VWM 2141 monitor (Pharmacia Biotechnology). Both columns were calibrated using commercial molecular mass (M_r) standards: Blue dextran 2000: $M_r = 2\,000\,000$, BSA: $M_r = 67\,000$; ovalbumin: $M_r = 44\,000$; lysozyme: $M_r = 14\,000$ and cytochrome *c*: $M_r = 11\,600$.

3. Results

3.1. Analysis of the conductivity of dispensed beer foam

The dimensions of the apparatus (internal diameter = 9.5 cm) and the volume of sample employed during this investigation were substantially larger than in previously reported studies on the conductivity of model foam systems [5] in order to facilitate generation of sufficient material for compositional analyses. An additional benefit of increasing the scale of the apparatus was a decrease in the area of vessel wall per unit volume of foam and thereby reducing potential interference by the vessel wall on foam collapse. The design of the conductivity probe also differed from previously reported studies and comprised two vertical stainless steel sensors which were located in the centre of the dispensed foam. The invasive nature of the use of the conductivity probe may be subject to criticisms in respect of sampling a relatively small proportion of the foam mass and influencing foam collapse characteristics. However, preliminary experiments with various inter-probe spacings gave similar results and suggested that conductivity measurements reflected actual events occurring within the foam rather than artefactual phenomena induced by the presence of the probe. Conductivity trials in which foam collapse was monitored either with retention or removal of the bulk beer gave similar results and demonstrated that removal of the bulk beer, and consequent lowering of the foam did not significantly alter foam collapse. These experiments also indicated that the reproducibility of results from conductivity analyses of dispensed beer foam could be enhanced by the inclusion of a surface conditioning stage by washing the vessel wall with sample prior to foaming. This enabled uniform generation of foam and prevented mechanical disruption of foam during collapse due to adhesion of regions of the foam in contact with the vessel wall.

The conductivity of dispensed beer foam declined with time in accordance with foam collapse. A comparison of the conductivity profiles of several commercial beers demonstrated similar gross features and suggested that for a given beer each profile could be divided into three regions termed initial, consolidation and residual stages of foam collapse, respectively (see Fig. 1 and Section 4). The initial fall in conductivity exhibited the characteristics of exponential decay as demonstrated by the linear relationship between the logarithm of conductivity and collapse time. A theoretical exponential conductivity decay curve was generated using the initial fall in conductivity to

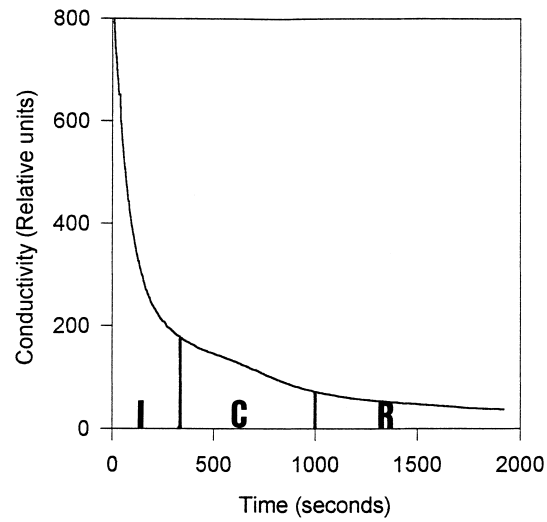

Fig. 1. A typical conductivity profile of the collapse of dispensed beer foam. The three stages of foam collapse are labelled: I, initial; C, consolidation and R, residual.

provide the value of the exponent k. A comparison of the theoretical and actual conductivity plots demonstrated that the duration of exponential decline in conductivity occurred from 0 to 360 s post-foam generation. This was followed by a stage characterised by a deviation from exponential decline (360–1000 s). The final stage in the conductivity profile of collapsing beer foam corresponded to residual conductivity resulting from a small persistent layer of foam.

3.2. Studies on the dynamics of liquid drainage from collapsing beer foam

Liquid drainage from batches of dispensed foam was continuously collected in fractions over 1 min time intervals (see Section 2). The weight of each fraction was recorded and this provided an accurate estimate of the rate of mass drainage from the foam. Plots of the natural logarithm of the initial drainage rate against time were linear, consistent with previous observations [6] (see Fig. 2). The value of the rate constant was estimated using the same procedure as described for conductivity plots and was used to construct a theoretical exponential plot for foam drainage. Experimental measurements of the rate of foam drainage showed good agreement with predicted values from 0 to 400 s but thereafter the rate of emergence of liquid from collapsed foam decreased from that predicted by the initial exponential decay. The yield of foam drainage material expressed either as a rate (Fig. 2) or cumulative yield (results not shown) followed exponential characteristics for approximately 85–90% of the total liquid collected from the collapsing foam over the duration of each experiment (0–30 min post foam generation).

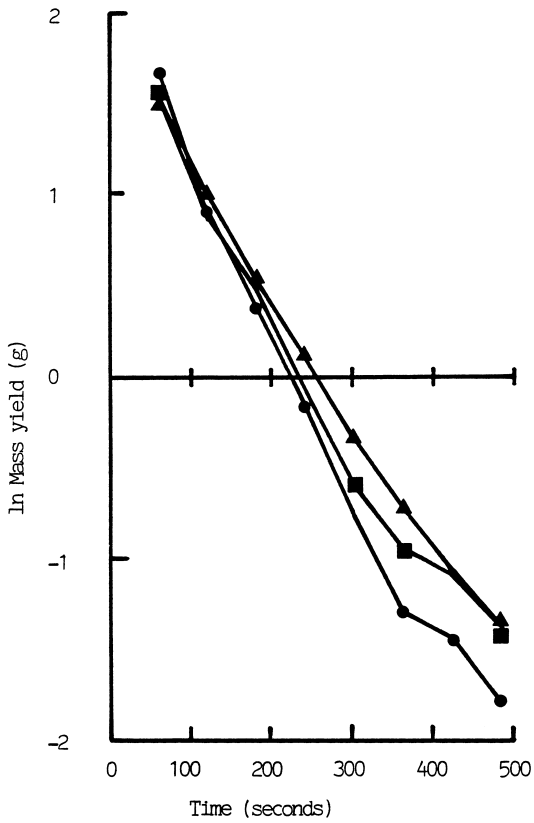

Fig. 2. The exponential characteristics of foam drainage during the initial stage of foam collapse. Data are shown for three replicate analyses.

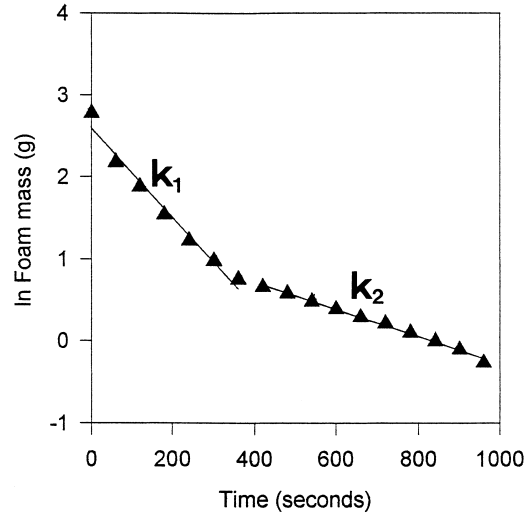

Fig. 3. Changes in the mass of material held within the foam (liquid + solutes + precipitated material) during collapse (k_1, k_2 correspond to the two exponential rates of mass loss in the initial and consolidation stages, respectively).

Changes in the total mass of liquid material held up in the foam were estimated by assuming that the initial beer content of foam corresponded to the summation of individual drainage yield measurements over the sampling period. This is a reasonable first approximation since the fraction of foam surviving 30 min post-foam generation was relatively small (estimated as <2%) when compared to the initial quantity of foam.

The decrease in the material held up in the foam was then estimated by subtraction of the known quantity of foam drainage material per sample interval. Plots of the decline in the total mass of liquid held up in the foam with time (Fig. 3) suggested that the decrease in foam mass deviated from the initial exponential decline after 300– 360 s and established a second and lower exponential rate. The initial exponential phase of foam collapse accounted for 85–90% loss of the total mass contained within the foam at generation.

3.3. Analysis of the solute composition of dispensed foam and foam drainage material

The solute composition of collapsed whole foam and foam drainage material collected during the initial exponential phase of foam collapse was identical to the unfoamed beer (Fig. 4). During the second stage of foam collapse, analysis of this material demonstrated a rise in

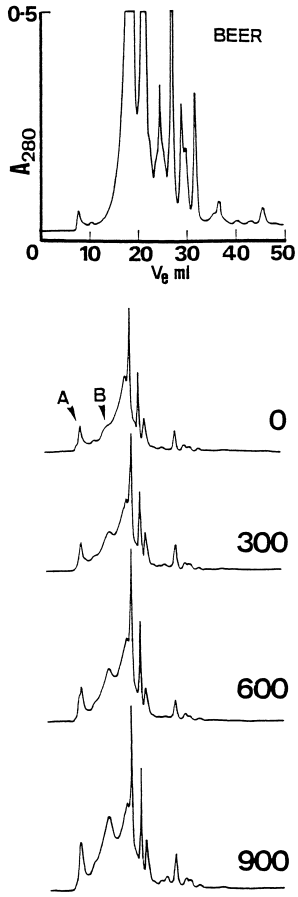

Fig. 4. Fractionation of acetone precipitated beer foam on a colum of Superdex 75. Time values (0, 300, 600, 900 s are quoted post-foam generation. Fraction A, $M_r = 70\,000$; fraction B, $M_r = 12\,000$ (M_r: molecular mass).

turbidity. A comparison of the Superdex 75 elution profiles of acetone precipitated foam (Fig. 4) demonstrated that fractions of relative molecular mass $M_r = 70\,000$ and 12 000 increased during the consolidation stage of foam collapse. Column chromatography (Superose 12) of precipitated foam solids responsible for turbidity confirmed the presence of polypeptide material and isomerised hop acids as reported in previous publications [6] (results not shown). Analysis of the amino acid composition of the high molecular mass material prepared by acetone precipitation of collapsed foam (see Section 2) showed an increase in total yield of amino acids (Table 1). However, analysis of the composition of polypeptide material demonstrated that the increase in the yield of individual amino acids was not uniform but varied between 1.3 to 2.9 fold increases in concentration (Table 1). The magnitude of this variation is greater than that expected from experimental error (±5%). The total content of hydrophobic amino acids (cysteine, valine, isoleucine, leucine, phenylalanine) increased proportionately more (2.2–2.9 fold) than hydrophilic amino acids (1.3–2.0 fold). These results was subsequently confirmed for both ale and lager samples.

Table 1
Amino acid composition (nmol/sample) of acetone precipitated material prepared from 500 μl collapsed beer foam at 0, 300, 600 and 900 s post-dispense

Amino acid	Foam sample post-dispense (s)			
	0	300	600	900
ASX	120	160	190	240
THR	77	89	100	130
SER	97	130	150	190
GLX	260	290	330	460
PRO	170	170	200	260
GLY	190	210	230	280
ALA	140	150	170	210
CYS	17	22	27	43
VAL	68	85	110	150
MET	22	25	30	41
ILE	34	49	67	89
LEU	52	74	100	150
TYR	33	39	46	60
PHE	21	24	32	45
HIS	68	77	78	92
LYS	76	84	96	110
ARG	80	90	100	130
Yield nmoles	1525	1768	2056	2680

4. Discussion

The experimental approach adopted in this investigation has attempted to integrate the results of physical and compositional analyses in order to understand the dynamic changes within foams and the accompanying material drainage from foams. Conductivity and compositional analyses of foam together with studies on foam drainage material suggested that foam collapse may be subdivided into three stages (initial, consolidation and residual). Analysis of the initial rates of decline in conductivity of dispensed foam and emergence of liquid from the foam both exhibited the characteristics of exponential decline with time and could be modelled by a simple exponential function. Thus

$$X = X_0 e^{-kt}$$

where X is the conductivity or rate of drainage at time t, X_0 the initial conductivity or drainage yield extrapolated to time 0 and k a constant.

Rudin [11] concluded that the relationship between volumetric foam drainage and collapse time was logarithmic only for a short proportion of foam collapse unless the sample was foamed to 325–350% in excess of the initial beer volume. In our experimental procedure, physical and compositional analyses were conducted on dispensed beer foam wherein the volume of liquid within the foam corresponded to a small fraction (25%) of the original beer volume. This relatively low value of volumetric foam fraction was selected to more closely approach actual dispense conditions. Nevertheless, in these systems 85–90% of the total mass of liquid, held within the foam at formation, had been lost via gravitational drainage during the first stage of foam collapse (0–360 s post-foam formation).

The second stage of foam collapse is characterised by a rapid decline in the rate of drainage from the foam. The conductivity of beer foam arises from both the liquid and solute content within the bubble lamellae. The deviation in the conductivity profile from exponential characteristics during this stage is probably caused by increases in concentration of polypeptide material within the foam as evidenced by compositional analyses (Fig. 4 and Table 1). This secondary stage of foam collapse was termed the consolidation stage to reflect the changes in foam characteristics such as density, yield of drainage material and solute composition. In contrast to the gravitational drainage of the primary stage of foam collapse the consolidation stage is more representative of foam stability. Conductivity measurements during this stage may provide a useful predictive indicator of foam stability in contrast to methodologies which exploit gravitational drainage of liquid rich foams (i.e. the initial stage of foam collapse).

The increase in the concentration of polypeptide material in both foam and foam drainage material during the consolidation stage may be explained in terms of the non-uniformity of solute distribution within the bubble lamellae. Selective partition of these surface active molecules to the gas–liquid interface and immediate sub-surface layers within the bubble lamellae occurs during foam formation. For example in studies on the behaviour of thin film model systems comprising solutions of single proteins the concentration of protein at the surface was estimated to be increased by a factor of 10^4 relative to the interlamella space [1]. Foam drainage during the primary stage of foam collapse removes liquid from between bubble lamellae that has the same solute composition as the bulk liquid. However, bubble thinning processes expose the surface and sub-surface layers that are preferentially enriched with surface active molecules. Drainage material from the consolidation stage of foam collapse thus contains elevated levels of polypeptide material and isomerised hop acids relative to the bulk liquid.

Two additional factors that may also contribute to the increase in solute concentration of foam and drainage material are evaporation of liquid from the thin films that comprise the foam structure and solid reinforcement of well drained foam by precipitated foam solids [12]. The latter feature would prevent liquid drainage from foam lamellae unless the contents were completely released by rupture processes.

The results from amino acid analysis of polypeptide material demonstrated an increase in the relative proportions of hydrophobic amino acids in well drained beer foam i.e. corresponding to the consolidation stage of foam collapse. This suggested that polypeptides of enhanced hydrophobic character, relative to the polypeptide composition of bulk beer, were selectively retained at the gas–liquid interface. This is consistent with several studies which emphasise the importance of hydrophobicity in the foam stabilising potential of beer polypeptide fractions [13]. However, it is unlikely that the partition selectivity of the foaming process is sufficient to implicate a single polypeptide as wholly responsible for the observed foam characteristics. The increase in the cysteine content of foam polypeptide material is contrary to previous observations [12] which reported a depletion in the cysteine content of foam polypeptide material. However, recent studies [14] have demonstrated that a polypeptide ($M_r \sim 10\,000$) that is probably derived from barley lipid transfer protein has eight cysteine residues (present as four disulphide bridges) and accumulates in beer foam. Detailed studies on the selective retention of polypeptides within foam are however considerably hampered by the complexity of the beer polypeptide composition as demonstrated by two-dimensional gel electrophoresis [15].

Estimates of the total mass of material held within the foam suggested a two stage exponential decay during foam collapse. Clearly the primary stage of foam collapse is driven by gravitational drainage from a liquid rich environment. The establishment of the second exponential rate (k_2) suggests a change in the mechanism of foam collapse during the consolidation stage. The results from compositional analysis of foam and foam drainage material suggest that the mechanism of foam collapse during this stage is based on changes within the foam structure due to disproportionation and bubble coalescence which ultimately results in bubble rupture [4]. Gravitational drainage and bubble rupture processes are not mutually exclusive and probably occur within the initial and consolidation stages of foam collapse. However, as bubble thinning proceeds in the upper part of the foam during stratification within the initial stage and with the onset of the consolidation stage, the principal driving force of foam collapse changes from drainage to bubble rupture. The release of surface adsorbed polypeptides and isomerised hop acids by bubble rupture in the primary stage would be effectively masked by the large excess of bulk liquid phase entrapped in the bubble lamellae. It is only when gravitational drainage of liquid is largely complete that the release of adsorbed material by bubble rupture processes becomes detectable in foam drainage material.

5. Conclusions

The results of conductivity measurements, in conjunction with compositional analyses, suggested that beer foam collapse can be divided into three stages viz initial, consolidation and residual stages. The first stage of foam collapse is dominated by gravity driven liquid drainage and accounts for the removal of 85–90% of the total mass (liquid + solutes + precipitated material) from the foam. The solute composition of the foam at this stage is indistinguishable from that of the beer due to the high levels of temporary entrapment of bulk liquid. The density of the foam rapidly decreases as liquid drains from the bubble

lamellae and leads to the consolidation stage. This is characterised by an increase in the concentration of polypeptide material in both the foam and drainage material as surface and sub-surface regions of the bubble lamellae enriched with surface active molecules are subjected to collapse processes. The final stage of foam collapse is characterised by a thin layer of residual foam. Thinning of foam lamellae results in a transition from gravitational drainage of bulk liquid to foam collapse proceeding by bubble coalescence, disproportionation and rupture processes during the subsequent consolidation and residual foam stages.

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References

[1] D.C. Clark, M. Coke, L.J. Smith, D.R. Wilson, Foams: Physics, Chemistry and Structure, Ch. 5, in: A.J., Wilson (Ed.), Springer, Berlin, 1989.

[2] C.J. Dale, T.W. Young, Rapid methods of determining the high molecular weight polypeptide components of beer, J. Inst. Brewing 93 (1987) 465.

[3] C.J. Dale. Ph.D. Thesis, University of Birmingham, 1989.

[4] E. Segal, P.R. Glenister, K.G. Koeppl, Tech. Quart. Master Brewers' Assoc. Am. 4 (1967) 104.

[5] D.C. Clark, P.J. Wilde, D.R. Wilson, J. Inst. Brewing 97 (1991) 169.

[6] C.J. Dale, S.G. Walker, A. Lyddiatt, Dynamic changes in the composition and physical behaviour of dispensed beer foam, J. Inst. Brewing 99 (1993) 461.

[7] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248.

[8] C.J. Dale, J.S. Hough, T.W. Young, Fractionation of high and low molecular weight components from wort and beer by adsorption chromatography using the gel Sephadex LH 20, J. Inst. Brewing 92 (1986) 457.

[9] C.J. Dale, T.W. Young, Low molecular weight nitrogenous compounds and their influence on the stability of beer foam, J. Inst. Brewing 98 (1992) 123.

[10] C.J. Dale, A. Lyddiatt, Quantitative analysis of purine nucleosides and free bases in wort and beer, J. Inst. Brewing 100 (1994) 173.

[11] A.D. Rudin, J. Inst. Brewing 63 (1957) 506.

[12] L.R. Bishop, J. Inst. Brewing 81 (1975) 444.

[13] P.T. Slack, C.W. Bamforth, J. Inst. Brewing 89 (1983) 397.

[14] S.B. Sorensen, L.M. Bech, M. Muldbjerg, T. Beenfeldt, K. Breddam, Tech. Quart. Master Brewers' Assoc. Am. 30(4) (1993) 136.

[15] C.J. Dale, T.W. Young, Fractionation of high molecular weight polypeptides by two-dimensional gel electrophoresis, J. Inst. Brewing 94 (1988) 28.