125th Anniversary Review: The Non-Biological Instability of Beer

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ABSTRACT

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Unlike many alcoholic beverages beer is inherently unstable. In chemical (as opposed to microbiological) terms this instability can be considered – and is here reviewed – in the categories of colloidal instability, foam, gushing, flavour instability and light sensitivity

Key words: Beer, bits, flavour instability, foam, gushing, haze, light-struck, precipitates.

INTRODUCTION

Beer is inherently unstable. Its properties change with time, either in a very short period such as during the drinking experience (e.g. foam collapse and the appearance of light-struck character) and over rather longer periods (e.g. haze development and flavour deterioration).

It is possible to classify beer instability into several types:

Biological
Physical (haze, turbidity)
Foam
Gushing
Flavour
Light-struck.

Biological instability, viz. the growth of micro-organisms in beer, is the subject of a separate 125th anniversary review. This paper reviews the current understanding of the other forms of instability. The author and others have provided reviews on haze[9,87,145], foam[43], flavour instability[12,13,15,16,154], gushing[29,46,131] and light-struck[40,150]. The present paper will highlight what the author perceives to be the most important facets of the literature in each case, with an emphasis on more recent findings which post-date earlier reviews.

Dedicated to Dr John R. Hudson, 1924–2011, who brought me into the brewing industry and whose wise counsel kept me there.

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PHYSICAL INSTABILITY

Also known as colloidal instability, we can sub-divide physical instability[9] into the categories of

Precipitates
Bits
Haze
Invisible haze.

Precipitates

Precipitates tend to develop when beer is subjected to extremes of temperature. One early report was of a precipitate comprising β-glucan that arose in a high alcohol beer that had been inadvertently frozen[48]. This report highlights the importance of low temperature in driving materials from solution[101]. With the exception of possibilities for freeze concentration and the less extreme "ice beer" technology, there is no practical opportunity to freeze beer in breweries to boost the removal from solution of colloidal materials (water swells when frozen and that would be undesirable if vessel integrity was to be maintained). Equally it is important that beer is not inadvertently frozen in transport and storage. Gjertsen's report[48], however, also highlights that it is not only protein and polyphenol that must be considered when discussing colloidal instability of beer.

At the other extreme, beer exposed to high temperatures may also develop precipitates. One such example was an alcohol-free beer heated to temperatures exceeding 50°C in a Middle Eastern market, developing a gelatinous precipitate as a result of the interaction between isinglass fining material and the foam stabilizer propylene glycol alginate[9].

Bits

If not noticed in package (perhaps because much beer is in cans as opposed to bottles), precipitates are still a problem if beer is subsequently dispensed into glasses, because they will disintegrate into bits, discrete particles suspended in beer which otherwise has a "bright" background.

Such bits are not always associated with precipitate formation and, indeed, may be very difficult to see clearly, which means that they do not always present an obvious problem as does distinct turbidity in a beer, which is expected to be bright. The surest way to detect bits is to filter the beer through a filter paper (perhaps a 3.2 cm di-

ameter paper) and stain with methylene blue. The method can be semi-quantified by having comparison papers in which different quantities of bits have been stained.

It is sometimes seen that bits are a problem associated with insolubilisation of additions made to beer (e.g. see above). Published examples include the famous case associated with the demise of the Schlitz brewery and that of papain cross-reacting with PGA during pasteurisation. However materials endogenous to beer can also be problematic: Walters et al.[160] reported the development of bits due to protein and pentosan that arose during the fobbing of beer inside packages as they were shipped around the globe. This illustrates a second truism: that agitation of beer exacerbates clarity problems.

Haze

"Haze proper", namely that which delivers a uniform lack of clarity to a beer, can arise from a number of materials (in addition to the growth of living organisms): starch[88], pentosans[36], oxalic acid[51] and of course protein-polyphenol complexes. Less common causes reported have been can lid lubricants[130] and dead bacteria mainly from malt[7,157]. Haze is customarily divided into "chill haze", which develops when beer is chilled to 0°C, but returns into solution when the beer is warmed to 20°C, and "permanent haze", which is present in beer at all practical temperatures. The extent to which visible haze is detectable by consumers and how significant it is concerning their preference has been investigated[32,132].

Invisible haze

Sometimes called "pseudo hazes"[74], these are due to very small particles (<0.1 μm) that cause high levels of light scatter when haze is measured at 90° to incident. Identified causes include tiny particles originating in unmodified regions of the starchy endosperm of barley[74], retrograded starch[163] and polysaccharides sloughed off the surface of yeast cells[89].

Proteins, polyphenols and colloidal instability

Outtrup et al.[112,113] highlighted that the most pertinent polypeptides in beer with regard to colloidal instability are those rich in proline and glutamine and which originate in the hordein fraction of barley. Outtrup also emphasized the importance of hydrophobic amino acid residues with regard to the growth of haze particles and it should be emphasised that there can be no absolute distinction between haze-potentiating and foaming polypeptides, the latter being noted for their hydrophobic character (see later). Ishibashi et al.[71] used immunological techniques to show that antibodies raised against haze reacted with proteins classified as haze polypeptides, but also those claimed to be foam-stabilizing.

Monomeric polyphenols, such as catechin, become haze-potentiating when they are polymerized through oxidation[98,111]. McMurrough et al.[97] suggest that dimers represent the most potent entities.

Siebert and Lynn[134,136] presented a model to explain the interaction of dimeric polyphenols and proline-rich polypeptides in the production of chill haze and noted that pH has a significant impact on these interactions[133].

Enhancing the colloidal shelf life of beer

The whole of the malting and brewing processes can be thought of as an exercise in diminishing the levels of materials in beer that will tend to come out of solution as hazes, bits and/or precipitates. Certainly adequate and homogenous malt modification is important if the risk of β-glucan-derived turbidity is to be minimized[8]. This can be augmented by the use of low temperature mashing-in protocols and possibly the use of exogenous enzymes, with combinations of β-glucanases and xylanases being especially efficacious[127]. Adequate calcium to eliminate oxalate problems is important[27]. Two critical stages for the removal of colloidally sensitive materials are a vigorous rolling boil[7] and cold conditioning[101]. Downstream, sensitive protein can be removed by silica preparations[96], tannic acid[105], papain[42] and prolyl endoproteinase[91]. Reduced input of haze-promoting polyphenols can be achieved by the use of low proanthocyanidin barleys[117], alkaline steeping of grain[38] or even dehusked grain[73]. Hop extracts are devoid of polyphenols[64]. Downstream, polyphenols may be removed by polyvinylpolypyrrolidone[109].

Predicting the colloidal shelf life of beer

A diversity of methods have been proposed and used in an attempt to forecast the physical shelf life of beer. They can be divided into methods that (a) measure specific haze components (b) "force" the beer, thereby accelerating the development of haze (and other elements of colloidal instability notably precipitates and/or bits). Clearly the first type of method has serious inadequacies if only one or a relatively few are performed. For example, one method may not reveal a beer to have a worrisome level of haze-forming protein – but that says nothing about its content of polysaccharides, oxalate and so on. For this reason, some brewers have based their predictive techniques on a combination of a pair of such methods, e.g. measurements of protein and polyphenol, but even that may be inadequate.

The second type of method is more reasonable, as (depending on its precise nature) it should assess the tendency of **all** colloidally-sensitive materials to "drop" out of solution. These methods can be divided into those that challenge the beer by extremes of heat or by hot-cold cycling and those that involve adding an agent (notably alcohol) that, allied to extreme chilling, will lead to any material that has a tendency to leave solution so to do.

In terms of the former type of method we can include:

i) for protein: the saturated ammonium sulphate precipitation limit (SASPL) test and the tannic acid precipitation test[23,26,128]
ii) for polyphenol: the colorimetric determination of total polyphenol, titration with polyvinylpyrollidone (PVP) and high performance liquid chromatography[135].

Amongst the forcing tests[108] are:

i) The European Brewery Convention (1963 method) in which beer is held at 60°C for 7 days then cooled to 0°C for 24 hours and the haze measured.
ii) The Harp method in which the beer is stored for 4 weeks at 37°C followed by 8 hours at 0°C and the haze measured.

iii) Various cycling methods, such as the one that holds beer for 24 hours at 37°C then for 24 hours at 0°C, this supposedly representing the equivalent of one month of storage at non-extreme ambient temperatures.

Perhaps of rather more value are tests in which colloidally sensitive materials are forced out of solution. The most famous of these is the Chapon test[30], in which a sample of beer is chilled to –8°C without freezing (added alcohol prevents freezing) and left for 8 hours before the chill haze is measured. This type of test is especially valuable because any material that displays a tendency to fall out of solution is likely to be detected in this test, which combines the very low temperature and the added precipitant (ethanol).

FOAM STABILITY

Aesthetics of foam

Most beer drinkers are inclined to prefer beer displaying stable foam[11], although there are national and regional differences[140,141] and a possible gender distinction with regard to the preference for seeing foam adhering to the side of the glass (cling, lacing)[121].

Foam physics

The achievement of stable foam on beer is dependent upon an understanding and application of best practice founded upon physics and chemistry.

Beers are supersaturated solutions of carbon dioxide, but nonetheless foam formation is dependent upon nucleation phenomena occurring[95], which will occur if there are particles in beer or scratches on the glass[118], but which can be induced by vigorous dispense, the use of glasses featuring nucleation sites and in-package devices such as the widget[86]. As foams comprising small bubbles tend to be more stable, efforts to generate small diameter bubbles in these nucleation events are important[14].

The production of foam represents a huge increase in surface area, which is counter to the force of surface tension[120]. That this collapse is delayed in beer is due to the presence of surface active molecules (see later) that enter into the bubble wall and form a framework that holds it together. Some of these molecules will also have a tendency to retard the drainage of liquid beer from the foam, which also contributes to the longevity of the head.

The most important physical event leading to foam decay is the collapse of bubbles, due to coalescence and (much more importantly) through disproportionation[120]. This is the passage of gas from a small bubble to a larger bubble, leading to the collapse of the former and the increase in size of the latter to unappealing proportions. This is the primary reason why a uniform distribution of small bubbles is desirable for enhanced foam stability.

Disproportionation is described by the DeVries equation

$$r_t^2 = r_o^2 - \frac{4RTDS\gamma}{P\theta}\ t$$

where r_t = the bubble radius at time t
r_o = bubble radius at the start
R = the gas constant (8.3 J K^{-1} mol^{-1})
T = absolute temperature (°K)
D = the gas diffusion coefficient (m^2 s^{-1})
S = the solubility of the gas (mol m^{-3} Pa^{-1})
γ = the surface tension
t = time (s)
P = pressure
θ = the film thickness between bubbles

This explains the enormous benefits that nitrogen gas (much less soluble than carbon dioxide) has for beer foam stability[10,14,28,104], remembering that nitrogen adversely impacts the flavour of many beers[63].

Foam chemistry

The huge increase in surface area that occurs when beer foams is in direct opposition to the force of surface tension, which drives water to occupy the lowest possible area for a given volume. Stable foam therefore depends upon the presence of surface active molecules that enter into the head to form a matrix that counters collapse.

Principal amongst these molecules are the polypeptides derived from grain[6, 146] and the bitter acids from hops[24]. In each case a principal feature that drives the molecules into the foam and which contributes to the stabilizing reactions is hydrophobicity, such that the more hydrophobic the protein[138] or iso-α-acid, the greater is its contribution to foaming. In the instance of the bitter acids, this means that the reduced iso-acids, tetra and hexa, afford extremely stable, albeit coarse, heads[43]. Minor hop resin components may also be important[139].

Of the hydrophobic polypeptides, the most studied have been Protein Z[66,78] and Lipid Transfer Protein 1 (LTP1)[142]. The importance of hydrophobicity was especially highlighted in the latter instance by the observation that LTP1 is not especially foam active when isolated from grain, but that its foaming abilities are greatly boosted by boiling, with the attendant denaturation and exposure of the hydrophobic interior[22]. In terms of protein Z, it seems that the Z4 component correlated with improved foam performance, whereas the reverse was observed for protein Z7[65]. It has been suggested that protein Z and an α-amylase inhibitor correlate positively with foam whereas yeast-derived thioredoxin was possibly foam negative[66,107].

The foam stability due to proteins reflects a balance between the respective levels of polypeptides derived from hordein and the albuminoid polypeptides Z and LTP1[17]. The former may have an enhanced tendency to enter into the bubble wall but, once there, they are not as foam-stabilizing as the albumins. Picariello et al.[116] used immunological approaches to confirm that hordein- and albumin-derived polypeptides can be found in foam. Wang et al.[161] confirmed that barley hordeins have good foaming capacity. It is also understood that proteins associated with carbohydrates are important for foaming[24,115]. Carbohydrate moieties attached to polypeptides lower the extent to which foaming polypeptides such as LTP are lost through the brewing process[85].

Polypeptides derived from wheat appear to have superior physicochemical properties as pertains to foaming[79]. It has long been recognized experientially that the inclusion of wheat in the grist benefits foam, and it is further claimed that mashing at increased temperatures[72] and

lower pH[57] is to the advantage of foam stability. Equally, it is understood that high gravity brewing is detrimental to foam stability[35], in part due to stress on the yeast causing the release of damaging proteolytic enzymes.

Other positive contributors to foam stability include Maillard reaction products[93], divalent metal ions such as zinc[121] and added foam stabilizers, notably propylene glycol alginate[76].

Nonetheless, it has been proposed that the majority of foam problems in the trade are not a consequence of a shortage of foam-positive materials, but rather the presence of foam negatives (inhibitors) such as lipids and detergents in inadequately cleaned glassware[10]. It has been proposed that it is generally the case that beers contain adequate foam-positive entities and deficiencies in the beer per se are more likely to reflect the presence of foam-negative substances[83].

Measuring foam stability

No single quantitative procedure can quantify all foam attributes.

Foam stability (head retention) historically has been measured by drainage methods based on a simple glass apparatus[2,34,122].

The method developed by Klopper[82] and marketed as instruments under the NIBEM trade name measures foam decay based on conductivity detection, while other commercial instruments photometrically measure drainage[4,19,119]. Good correlations were observed between the values determined by diverse methods[158].

Simplest of all are the methods based on shaking[80], whilst at the other extreme of complexity are those employing video imaging[44] and scanning electron microscopy[58] to assess parameters such as bubble-size distribution.

Lacing can be assessed by gauging surface coverage of foam photometrically[84] or by the lacing index procedure, where laced foam is collected and quantified by ultra violet light absorption[75].

Comparison of beers for their ability to generate foam is possible using a nucleation method[95].

GUSHING

Despite it being a supersaturated solution of carbon dioxide, beer does not spontaneously erupt into foam unless there is a nucleation phenomenon at play. If a powerful nucleation centre is present in beer, then this can lead to an unwanted immediate foaming once a container is broached[49]. Most prominent amongst these gushing promoters is the intensely hydrophobic polypeptide hydrophobin[165], sourced from fungi such as Fusarium that can contaminate grain[144]. In recent times, lactic acid bacteria have been deliberately seeded into malt houses to overcome the growth of Fusarium[92]. Other gushing potentiators include hop resin degradation products[1], oxalate[129], filter aid breakthrough[81], metal ions[52], tensides[47], uneven carbonation[164] and of course agitation.

FLAVOUR INSTABILITY

Probably the most challenging quality problem that remains for brewers is the achievement of flavour stability. However, debate often centres on whether this is more of an issue for the brewer than it is for the consumer. It was found that brand identity has a major impact on selection preferences, apparently rising above the extent to which a given beer displays aged character[147]. Others have shown that imported beers tend to be preferred to domestic ones, the selection being clearly made on a perceived superiority of such beers, despite the fact that those very beers display aged characteristics deplored by brewers[53]. It truly could be argued that a consumer can see quite clearly, for instance, whether a beer is "bright" or whether it displays inadequate foam performance, but aromas that professional brewers often regard as unacceptable might be preferred or, at the least, ignored by drinkers.

Assuming, though, that the goal of every brewer should be to minimize flavour change in a product, the challenge is manifest. It can be fairly argued that *any* change in aroma or taste represents flavour instability[15]. As there are literally hundreds of molecules in beer that might change in level in amounts at or above their flavour threshold, it is a far more complex problem than, say, ensuring that the relatively limited number of colloidally-unstable molecules are depleted. Indeed, the flavour thresholds of many substances in beer are remarkably low, for example E-2-nonenal has a flavour threshold of approximately 0.1 ppb.

For this reason it is a more logical approach to adopt procedures which minimize changes in the level of *all* flavour active molecules in beer[12]. Such generic approaches fundamentally are reduced to eliminating oxygen and its reactive variants, reducing temperature and incorporation (where permitted) of antioxidants and binding agents, of which sulphur dioxide is the most prominent[67,68].

Temperature has a huge impact on the flavour stability of beer and it has been stressed that for every 10°C increase in temperature, then the rate of chemical reactions leading to flavour change in beer is increased between two to three times[12].

Before discussing these changes, it is also important to draw attention to the difficulty of drawing firm conclusions from much of the extant literature. As highlighted by Meilgaard[100], the number of sensory studies in this field that pass critical examination are few indeed. Furthermore, many of the studies reported on flavour stability assess differences between trial and control brews on the basis of *intensity* of aged notes. Whilst this is not unimportant, far more relevant is measuring the time taken for the first appearance of a flavour change, whether the loss of a note or the appearance of a note[12]. This is common sense: for a phenomenon that we talk about in terms of time ("what is this beer's shelf life?"), we should surely quantify it primarily on the basis of units of time.

Too often, attention is paid to relatively few flavour notes associated with ageing and, of these, cardboard or wet paper is the most frequently cited. This is hopelessly limiting, all the more so when the only chemical entity cited is E-2-nonenal. Whilst important in ageing (although not always[155]), E-2-nonenal is just one of numerous chemical species that must be considered (see[154] for a comprehensive list). Saison et al.[125] have narrowed the list

somewhat, suggesting that cardboard flavour was primarily linked to (E)-2-nonenal. They also confirmed that methional, 3-methylbutanal, 2-furfuryl ethyl ether, β-damascenone and acetaldehyde are key contributors to aged flavour, with (E,E)-2,4-decadienal, phenylacetaldehyde, 2-methylpropanal, diacetyl and 5-hydroxymethyfurfural having somewhat lesser roles. It was stressed by Vanderhaegen et al.[153] that different beers age in different ways and that the importance of separate chemical reactions in this context changes, for example from pale lager beers to those containing specialty malts.

Perhaps too many conclusions regarding factors that impact flavour stability have been made on the basis of chemical or physical measurements rather than on organoleptic analysis, which is perforce the ultimate gauge of whether a process ingredient or stage has an impact on flavour. Prominent amongst these analytical procedures is the use of electron spin resonance spectroscopy (ESR)[152]. One interesting recent development is the peroxide challenge test, which gauges a beer's ability to quench hydrogen peroxide[102]. The greater this capability, the greater the flavour stability as gauged both by ESR and organoleptically.

There is no questioning that oxygen levels in packaged beer should be as low as possible if elongated shelf lives are to be achieved. It is now recognized that air can leak into bottles at the crown cork-neck interface[62], driving some brewers to revert to pry off crown corks and to investing in oxygen-scavenging crown corks[151]. Nevertheless, it is clear that beer in a can does not suffer from air ingress and neither does a can allow light to encroach. Perhaps the worst small pack medium remains plastic bottles, despite recent developments in materials whereby air ingress through the container wall is now less than in earlier plastic formulations[59].

The debate has centred for some years now on the extent to which oxygen exposure earlier in the process is detrimental to flavour stability. Some believe that oxygen ingress throughout brewing is important whereas others have concluded that air uptake upstream of the fermenter is an irrelevance[110].

Those advocating minimum oxygen ingress in the brewhouse invoke inter alia the enzyme lipoxygenase as being a primary catalyst for the oxidation of unsaturated fatty acids, eventually leading to the production of unsaturated carbonyl compounds (such as E-2-nonenal) with their pronounced cardboard flavours[156]. Barleys devoid of the capability of making this enzyme have been bred[137] and claimed to benefit shelf life[61].

It has been observed, however, that even in the absence of lipoxygenase, unsaturated fatty acids can be degraded through the action of reactive oxygen species[20]. Oxygen activation is effected by metal ions, notable iron and copper[18], although it has recently been suggested that manganese, derived from malt, may be at least as relevant[168]. Irrespective of whether oxidation of unsaturated fatty acids is enzyme-driven or non-enzymic, the minimization of oxygen levels in the mash would be beneficial. Notwithstanding, it stands repeating that there is no unequivocal published data that establishes absolutely that efforts to lessen oxygen uptake in the brewhouse benefit the flavour life of beer[12].

The importance of yeast for its ability to produce SO_2[67] and also its predilection to reduce the carbonyl substances responsible for aged character[114] has been stressed recently[123,124,126]. Saison et al.[123,124,126] found that volatile aldehydes were removed almost entirely by yeast in fermentation, again drawing into doubt the significance of upstream oxidation from a perspective of flavour instability. It has been stressed that there is no advantage in oxygenating yeast directly to avoid aerating wort prior to pitching – the short contact times of oxygen with wort are insignificant[41]. Others have drawn the opposite conclusion[99]. Maillard reaction products inhibit lipoxygenase[143].

Apart from unsaturated fatty acids, other potential precursors of aged character in beer include iso-α-acids[56], higher alcohols[54] and amino acids through the Strecker degradation[55]. It has been suggested that the last of these primarily occurs in wort production, and that lower thermal loads would lessen its occurrence[149]. Cortes et al.[37] suggest that organic radicals, produced during roasting of specialty malts, provoke increased oxidation in mashing and more radical production during boiling. In turn this leads to reduced antioxidant and sulphur dioxide levels in the finished beer. This study seemingly contradicts other studies that suggest that Maillard reaction products are important antioxidants[31, 69].

Apart from leading to aldehydes that contribute to papery character, the degradation of the bitter acids can lead to the development of lingering harsh bitterness[70]. Trans isomers are less stable than cis isomers[39]. The α-acids and β-acids are more potent radical quenchers than are the iso-α-acids and polyphenols[166]. Aldol condensation interactions between different carbonyl compounds can lead to different carbonyl substances[55].

Sulphur dioxide undoubtedly provides a substantial opportunity to enhance the shelf life of beer, either as an antioxidant per se[3] or through its ability to bind the unsaturated carbonyl compounds responsible for aged notes[21]. However, there is a reluctance to use it in markets such as the US, as it must be declared on the label if present in quantities greater than 10 mg/L. Modified lager strains with enhanced SO_2 production without increased hydrogen sulphide levels have been described[167]. Other antioxidants native to the raw materials of beer include ferulic acid[159], colouring agents including Maillard reaction products[148] and polyphenols[5]. It has been reported that the mode of hopping is very significant in respect of the delivery of hop antioxidants into beer[103]. It has even been suggested that adding hop leaves to the brew kettle can suppress radical formation[60]. Enzymes have been suggested as aiding the protection of wort and beer from oxidation, including superoxide dismutase[33], glucose oxidase[106] and catalase[60]. Fredericksen et al.[45] suggest however that superoxide dismutase and catalase are limited in their ability to restrict radical production in mashing. Glutathione has been nominated as the main antioxidant found in beer[162]. Ascorbic acid is generally considered not to be especially relevant as an antioxidant in a beer context, but Jeney-Nagymate and Fodor[77] suggest that its addition alongside, surprisingly, the water-insoluble α-tocopherol (vitamin E) to cooled wort prior to pitching, benefits shelf life as gauged by ESR.

LIGHT-STRUCK

Exposure of beer to visible and ultra-violet light leads to the degradation of iso-α-acids and the production of 3-methyl-2-butene-1-thiol, and the (for many but not all[147]) reprehensible aroma of skunk[150]. It is now understood that additional substances are produced in the light struck reaction[94]. Brown glass largely (but not entirely) protects against the ingress of light at these wavelengths (350–500 nm), but clear and green glass afford no or little protection. For those intent on packaging beer in such glass containers, one defence against the light-struck reaction is the use of reduced iso-α-acids, which do not degrade to the compounds with the skunk character[50]. An alternative strategy might be to seek to eliminate riboflavin from beer, as it is this substance that transfers light energy into the skunking reaction[25].

CONCLUSION

Much is known about the myriad factors that lead to the instability of beer. When we consider microbiological contamination, foam, physical instability, gushing and light sensitivity, it seems that, for the most part, the understanding of many of the key issues is relatively comprehensive and that reliable strategies are now in place to minimize them as major problems. It is flavour instability that remains the severest challenge.

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