125th Anniversary Review: Improvement of Higher Gravity Brewery Fermentation via Wort Enrichment and Supplementation

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ABSTRACT

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Intensification of the industrial brewing process, particularly the use of higher gravity worts, has been driven by increasing competition within the industry as well as the need to maximise the use of raw materials and minimise energy expenditure. These developments have, however, placed greater demands on brewing yeast strains, whose evolutionary history has not prepared them for the extreme conditions associated with higher gravity brewing. Various yeast nutrient supplements have been used or proposed to maintain yeast performance under stressful conditions. These have included specific metal ions, lipids and lipid components such as fatty acids and sterols and free amino nitrogen, usually supplied in the form of a complex yeast food. Correction of wort nutritional deficiencies may reduce stress sensitivity of yeast and improve fermentation performance. Potential negative consequences of altering wort composition must however be considered, as important beer quality attributes such as taste, stability and foam can be affected. Here, the various options for nutrient supplementation and their influence on yeast physiology and performance, as well as beer characteristics are considered.

Key words: high gravity, nutrition, supplementation, wort, yeast.

INTRODUCTION

The use of high gravity wort (16–18°Plato) has become common practice in many breweries. The success of this system has been due to increased production capacity, with benefits including reduced energy input and reduced labour, reduced cleaning and waste costs and possible improvements in beer flavour[193]. In addition, the introduction of high gravity fermentation requires no extra brewery facilities and therefore no major capital investment is required[193]. Fermentation of even higher gravity worts would likewise reduce costs and contribute to the sustainability of the process. While some brewers are routinely operating with wort at 17.5–18°P, pilot-scale trials have demonstrated the feasibility of fermenting very high gravity (VHG) worts (20–25°P) without major detriment to beer flavour characteristics[36,139]. The use of worts at this gravity has not, as yet, been adopted by the brewing industry for the production of standard strength beers. Fermentation with VHG worts has been associated with reduced fermentation rates, a disproportionately high production of esters[7,96,163,233], extended lag phase duration[163], foam instability[44], increased concentrations of residual sugars in beer[163,193] and generation of yeast crops with poor fermentation potential[32]. The implementation of VHG wort fermentations at an industrial scale will almost certainly require optimisation of a number of parameters (yeast strain tolerance and performance, process parameters, etc.) Here, we review the literature relating to the possibilities for improved fermentation performance through wort supplementation with metal ions, lipids and 'yeast foods' and consider the impact of such additions on beer character.

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METAL IONS

Zinc

It has long been known that zinc has a crucial role in wort fermentation and Zn is the most commonly utilized supplement in the brewing industry. Brewery wort can contain between 0.1 and 5.0 ppm Zn and concentrations are dependent on a number of factors including the malt used[97], wort preparation and composition[94,95,111,120]. Wort Zn concentration represents only a fraction of the Zn present in malt and most Zn is retained with the used grains[11] or is lost through protein precipitation and wort clarification[94,120]. In wort, Zn may be rendered unavailable for biological use through its association with amino acids[94]. Even exogenously supplied Zn may have limited availability depending on when it is added to wort, with addition at time of pitching appearing to be optimal[203]. Consequently, worts are commonly Zn-deficient, a factor which in the past may have been ameliorated by the presence of galvanised components within the brewery vessels and pipes. The predominance of stainless steel in the modern brewery and 'dilution' of all-malt worts with sugar adjuncts results in an increased requirement for Zn supple-

mentation. Zn has multiple roles to play in yeast biology, acting as a structural or catalytic co-factor for a number of proteins, including enzymes associated with glycolysis and alcohol synthesis[56,130]. Zn deficiency results in the differential regulation of tens of genes in both shake flasks[85,130] and chemostat cultures[54] and approximately 3% of the yeast proteome requires Zn to function normally[65]. Interestingly, De Nicola and co-workers[54] found that the genes whose transcription were regulated by Zn availability had varied biological roles, including the metabolism of storage carbohydrates and the biogenesis of mitochondria, as well as flavour development through the regulation of branched chain amino acid synthesising genes. Zn also has an important role in processes involving Zn-finger DNA-binding proteins[25] such as Msn2p and Msn4p, which regulate the general stress response[135]. Furthermore, Zn appears to play a role in maintaining the redox balance within cells and its limitation can result in overproduction of reactive oxygen species (ROS), potentially resulting in DNA damage, necessitating an antioxidant response in the yeast cell[229].

The importance of Zn during brewery fermentation is reflected in the relatively rapid uptake by yeast cells in the first hours of fermentation[31,53,55,122,165]. Zn is first adsorbed to the cell wall and subsequently imported into the cell, where it accumulates in the vacuole[55]. Wort Zn levels are variable but a value of 0.2 ppm Zn results in optimum fermentation time in standard gravity (ca. 12°P) worts[31,84,93], and values lower than 0.1 ppm typically result in sub-optimal fermentation. Zn supplementation increases yeast growth[132] and improves fermentation rates[31,93,39,189,214]. A number of factors are, however, known to influence the requirement of yeast for Zn during fermentation. Helin and Slaughter[84] found, for example, that the requirement for Zn was influenced by the concentration of Mn ions in the wort, with 0.6 ppm Zn having an inhibitory effect on fermentation when wort Mn levels were low (<0.01 ppm). Brewing yeast performance is, however, known to be unaffected by Zn concentrations as high as 500 ppm[71]. Similarly high levels of Zn tolerance have been observed in other studies and have also been associated with Mn availability[102,173]. Rees and Stewart[173] reported that viability of yeast strains was in many cases not decreased in the presence of 65.5 ppm wort Zn, and a concentration of 327.5 ppm did not affect viability significantly in some strains. The same authors noted that increasing the Zn content of the fermentation wort led to reduced attenuation time, increased uptake of fermentable trisaccharides and increased ethanol production by lager strains[173]. An improvement in fermentation performance was generally seen up to 65 ppm, though in some cases a Zn concentration as high as 1,300 ppm was found to have a positive effect on fermentation. Generally, lager strains were better able to cope with excessive levels of Zn than were ale strains, though strain-specific differences were also observed within the lager strain group. Interestingly, the toxic effects of excess Zn were less pronounced at very high gravity (20°P), possibly due to a lower level of Zn in the original wort or the availability of Mn[173]. Ion-ion interactions are not however limited to Mn and other metal ions including those of calcium, magnesium, potassium and sodium have also been found to influence the effect of Zn on yeast fermentation performance[39,214]. These effects may be related to the ions' competition for binding sites on chelating molecules such as wort amino acids and peptides.

While Zn can have a toxic effect on yeast, the concentrations found naturally in worts are unlikely to be high enough for this to occur. Rather, wort Zn may act as a stress protectant. Improvement in ethanol production with increasing Zn supplementation[39,173] may suggest that Zn has a role in protecting cells against ethanol toxicity. Addition of Zn to growth media has been shown to protect yeast cells against subsequent ethanol shock in the form of a 30-minute exposure to an 18% (v/v) ethanol solution, with a value of 0.02 g/L Zn sulphate (8 ppm) being optimal[230]. This result was confirmed in further studies that showed that ethanol tolerance and synthesis were improved in Zn-supplemented media and that the greater resistance to ethanol was associated with increased production of trehalose and ergosterol[234], which are known to protect membranes against ethanol-induced damage[118,134]. The fact that the ethanol-resistant cultures were also resistant to heat shock supports the idea that resistance was brought about through membrane protection, as both parameters are known to affect membranes in a similar manner and membrane integrity is a main determinant of heat and ethanol stress resistance[78,164]. The mechanism by which Zn protects cells from ethanol toxicity is not fully understood, but could conceivably involve direct interaction with cellular membranes[21] and alteration of membrane fluidity (i.e., rigidification of the membrane to offset the fluidifying effect of ethanol). The antioxidant properties of Zn are well-known, particularly in relation to oxidative stress in animal cells, with possible antioxidant mechanisms including protection of sulfhydryl groups from oxidation, prevention of hydroxyl radical generation through antagonistic effects on Fe and Cu ions and support of proper functioning of the antioxidant enzyme Cu/Zn superoxide dismutase[166,216]. Zn deficiency in yeast cells is also known to result in oxidative stress through the intracellular production of ROS and Wu et al.[229] have shown that the *TSA1* gene, encoding cytosolic peroxidase, is activated under Zn-deficient conditions. Zn supplementation was also required to repair growth defects observed in a *Δtsa1* mutant strain[229]. The antioxidative properties of Zn may be particularly important during higher gravity fermentation, as ethanol is known to elicit an antioxidant response in yeast[4,215] and yeast strains with a compromised antioxidant potential are known to be hypersensitive to ethanol stress[45,46,160]. The importance of antioxidants in protecting cells against ethanol toxicity is also shown by the fact that Mn superoxide dismutase has an important role in protecting cells against ethanol toxicity, most likely due to the location of this antioxidant enzyme in mitochondria, the main source of ROS in cells[80]. ROS production in response to ethanol has even been observed under hypoxic conditions[115], suggesting the possibility of similar reactions occurring during brewery fermentation.

While *in vitro* studies have identified effects of Zn on yeast flocculation properties, it is unlikely that these effects will be apparent during wort fermentation. Taylor and Orton[207] have, for example, noted that 0.1 M Zn chloride (6,540 ppm Zn) inhibits flocculation in a buffer solu-

tion at pH 7.6. Raspor and co-workers[170] have observed enhanced flocculation up to a concentration of 2.6 ppm, with reduced flocculation occurring thereafter, though this was only observed with *Saccharomyces cerevisiae* and *S. diastaticus*; no effect whatsoever was observed with a lager strain. Conversely, Wackerbauer et al.[220] found that propagation of yeast with Zn supplements subsequently led to enhanced flocculation during fermentation, most probably as a result of increased growth rate and more rapid depletion of sugars from the system.

Given the relatively low concentrations of naturally-occurring Zn in wort and its low level of redox activity compared with other metal ions such as Cu and Fe[236], it is unlikely that Zn ions will have significant direct effects on beer flavour. The effect of Zn supplementation (up to 0.5 ppm) on yeast metabolism does however lead to significant changes in the synthesis of higher alcohols and esters[126,168,187,189]. Zn supplementation has been found to result in increased synthesis of isobutanol[126,187] and amyl alcohols[126,168], including isopentanol[187] and 3-methyl-1-butanol[189]. Ester synthesis is also stimulated by exposure to increased Zn concentrations, with increased concentrations of ethyl butyrate, isobutyl acetate, isopentyl acetate, ethyl hexanoate[187], ethyl acetate and isoamyl acetate[189,168] observed in fermented wort. Seaton et al.[187] attributed increased ester synthesis to changes in carboxylation, while Skands et al.[189] suggested that increased higher alcohol synthesis (and hence increased ester synthesis) was related to higher alcohol dehydrogenase activity, a result which would also explain the reduced levels of acetaldehyde observed in that study. Similar results have been observed when cells have been pre-conditioned to have higher concentrations of cellular Zn[53]. In a study to determine the effect of Zn pre-conditioning on flavour attributes of distillates produced from fermented distillery malt worts, De Nicola et al.[53] found a general increase in higher alcohols, though ethanol concentration was reduced by up to 1.8%. It is not clear why increased cellular Zn levels do not promote ethanol production by the Zn-dependent alcohol dehydrogenase. In the same study total ester levels were decreased by 25 or 38%, depending on yeast strain used.

Considering the relationship between amino acids and ester synthesis, it is also possible that the reduced amino acid uptake associated with Zn deficiency[126] will also result in altered flavour profiles. If extra Zn supplementation is to be carried out in an effort to improve yeast ethanol tolerance or productivity during higher gravity fermentations, the potential effects on ester synthesis and overall flavour profile of the finished product must naturally be considered.

The available evidence suggests that the influence of Zn on foam properties is, like the influence on flavour, indirect. Evans and Sheehan[68] reported an unpublished study by Evans and Stewart, which showed a positive correlation between the concentrations of various metal ions (including Zn) in malt and foam stability. It was suggested that the effect may be due to an alteration of malt protein content or an increase in wort Zn content (and yeast vitality) with a subsequent reduction in the release of foam-negative molecules such as proteases. The effect of metal ions on foam stability was found to be related to the malt ion content and not the beer ion content in that study, suggesting that ions were not stabilising foam through cross-linking with iso-α-acids[89].

Magnesium

Concentration of wort magnesium typically falls in the range of 50–90 ppm and appears to be directly related to concentrations found in malt. This relationship is apparently due to the high extractability of Mg (up to 80%) compared to that of other metals[94]. Mg content of cells peaks early in fermentation and is thereafter decreased as cell mass increases and Mg is effectively diluted[165].

Yeast cells have an absolute requirement for Mg, which acts as a cofactor for many enzymes and is necessary particularly for enzymes involved in glycolysis[224]. Efficient conversion of sugar to alcohol is therefore dependent on an adequate supply of bioavailable Mg. This is of critical importance for brewery fermentations, where worts typically contain sub-optimal levels of Mg and where Ca ions may have an antagonistic effect on Mg ion uptake. Walker and Maynard[225,226] have shown that Mg is also required for cellular growth and division in batch culture under glucose repressed conditions and increased growth rate in chemostat culture is associated with increased cellular Mg levels[225,226]. Increased uptake of oxygen and increased ethanol production also suggested an increase in respiro-fermentative activity in yeast cultures incubated with increased Mg[225].

Mg is cited as one of the most important ions present in brewery wort in terms of fermentation performance. Supplementation of both standard (12°P) and high gravity (20°P) worts with Mg (500 ppm) results in higher fermentation rates, increased uptake of maltose and maltotriose and increased production of ethanol, with up to an extra 5 mL ethanol L^{-1} produced during high gravity fermentation when Mg had been added as a supplement[172,174]. This promotion of fermentation performance by Mg was observed for both ale and lager strains[172,174] and later with wine yeast fermenting grape must. It is probable that most brewery worts do not contain optimal levels of Mg, and even when wort Mg was increased from a basal level of 75 ppm to 135 ppm, improvement in 16°P wort fermentation performance with a lager yeast strain was not observed[31]. An alternative to wort supplementation is the 'pre-conditioning' of yeast cells by propagation in Mg-rich wort. The Mg-rich cells produced have greater ethanol productivity in subsequent wort fermentations than their 'non-conditioned' counterparts[223]. Such pre-conditioning can increase cellular Mg levels by several-fold, depending on the Mg concentration of the propagation medium and duration of exposure[191]. Much of this Mg may be released when cells are pitched into fresh fermentation wort and this phenomenon has been proposed as a measure of the potential fermentation performance of a pitched yeast[147]. When considering the potential of Mg to improve yeast fermentation performance, the concentration of wort Ca should also be taken into account. Ca and Mg act antagonistically and any observed improvement may be related to the Mg:Ca ratio rather than the absolute concentration of Mg. Rees et al.[172] reported faster fermentation rates at Mg:Ca ratios of 17:1 and 11:1 for 12°P and 20°P worts, respectively, compared with unsupplemented

worts with Mg:Ca ratios of 3:1 or 2.5:1. Bromberg et al.[31] did not observe an improvement in fermentation performance with higher Mg relative to Ca, though in that case the highest ratio present in wort was 4:1.

Mg ions can positively influence flocculation and at a concentration of 10 ppm may substitute Ca under experimental conditions[195]. This characteristic may be relevant to brewery fermentation where the wort Mg concentration can be several fold greater than that of Ca[172]. Promotion of flocculation in a lager strain by Mg occurred between 12.5 ppm and 25 ppm Mg and was reversed when the Mg concentration was raised to 2,500 ppm. Increased flocculation of cells from a top-fermenting ale strain was observed at 2,500 ppm Mg[51]. The deflocculation of lager strain cells at 2,500 ppm Mg may be due to competition of Mg for the Ca-lectin binding sites[51]. Mg-induced flocculation is more sensitive to the presence of metal-chelating agents than Ca-induced flocculation and it is likely that the greater specificity of Ca in yeast flocculation relates to a greater affinity for cell wall-binding sites[51,198]. Smit et al.[190] reported that onset of flocculation was triggered in a lager strain by the depletion of any of a number of different nutrients from the growth media. The exception was Mg, the loss of which did not result in flocculation, suggesting that nutritional Mg has a role in the process. This role is not fully understood but may relate to cell surface hydrophobicity, which is reduced in cells grown in Mg-limited media (0.7 ppm). Inclusion of Mg in the assay medium did not increase the hydrophobicity (or flocculation potential) of the cells in that study[190].

Apart from the metabolic requirements of yeast for Mg during fermentation, these ions also have a well-documented role in protecting yeast from the toxic effects of ethanol and may therefore influence fermentation performance during higher gravity brewery fermentations. Mg supplementation reduces the toxic effect of ethanol in defined media. Walker[222] reported viabilities of 0% and 53% in a wine strain exposed to 10% ethanol for 24 hours in the presence of 50 ppm and 500 ppm Mg, respectively. Likewise, Hu et al.[88] showed that 9 hours of exposure of yeast cells to 20% ethanol led to the death of all cells in a population, whereas supplementation of the same medium with 85 ppm Mg resulted in viabilities of between 25 and 55% depending on duration of Mg exposure. Increasing Mg concentration led to increased resistance of brewing and distilling yeast to the toxic effects of ethanol[222]. It was proposed that Mg exerted its protective influence on the plasma membrane and, interestingly, Mg also protected cells against the effects of heat shock[222]. Ethanol and heat shock affect cell membranes in a similar manner[164] and Mg may therefore have a general protective effect on the yeast cell plasma membrane. Increased membrane permeability in response to ethanol exposure is well documented[40,75,103,169,182] and the protective effect of Mg has been attributed to its ability to prevent excessive loss of ions[162] and nucleotides[88] from the yeast cell. The presence of Mg obviates the requirement for heat shock protein synthesis, which would otherwise be needed to maintain plasma membrane integrity on exposure to ethanol[23]. Increased resistance to ethanol is conferred regardless of whether Mg is included in the yeast growth medium as a pre-conditioning step or whether it is added as a supplement concomitant with ethanol exposure[23,88], raising the question of how exactly it acts to maintain plasma membrane integrity under stressful conditions. Increased availability of Mg during higher gravity brewery fermentations may also benefit fermentation performance by mitigating the effects of osmotic stress on the cells[47].

Improvement of fermentation performance and stress tolerance of brewing yeast occurring as a result of Mg supplementation is clearly an attractive approach to increase productivity of higher gravity brewery fermentations. The potential negative effects of Mg on flocculation should however be considered, as well as the potential of these ions to influence the uptake of other essential metals such as Mn[24] and the ultimate effect of Mg supplementation on beer quality indicators.

Calcium

Jacobsen and Lie[94] reported malt grist calcium concentrations ranging from 180 to 1,600 ppm. Such high variability is typical for Ca and is likely due to variations in for example, soil conditions, fertilizer use and steeping liquor used in the production of the malt. The amount of Ca that is transferred to wort is variable (approx. 25 – 50% of malt levels or 15 – 35 ppm) and appears to be related to the solubility of the malt Ca, rather than the total concentration[94].

Ca ions have a role in protecting cells against the toxic effects of ethanol in non-complex media. Ca supplementation (30 ppm Ca^{2+}) improved sugar utilization, ethanol production and viability of *S. bayanus* fermenting a 21% glucose medium[151]. Likewise, Ciesarová and colleagues[41] found that fermentation in the presence of 10% ethanol was optimal with 3 mM Ca. The positive effect of Ca supplementation was manifested as improved ethanol production rather than improved growth[41], though a study by Lotan et al.[128] showed a clear stimulatory effect of Ca (up to 500 ppm) on growth of *S. pastorianus* in minimal medium. An increase in cellular Ca is also associated with bud emergence in yeast grown in minimal medium[179]. Carbohydrate starved cells show a transient increase in cytosolic Ca, which was rapidly compartmentalised when glucose was supplied[104]. Furthermore, subcellular distribution of Ca is known to be influenced by osmotic stress, with vacuolar stores of Ca released into the cytoplasm via a vacuolar membrane transporter when yeast are exposed to a hypertonic solution[129]. The importance of intracellular compartmentalisation of ions for brewing yeast cell homeostasis during higher gravity brewing is not known, but may explain strain-dependent fermentation performance in these worts.

Any positive effect of Ca supplementation on yeast growth is compromised by its antagonistic effect on Mg uptake and function[183]. The physiological demand for Mg is greater than that for Ca[102] and an increased concentration of wort Ca is likely to exacerbate Mg deficiency through antagonistic interactions[172,174,197], particularly since Ca may replace Mg in a number of biochemical pathways to the general detriment of the cell. Pyruvate decarboxylase activity, for example, is reduced when Ca is substituted in place of Mg[67]. Ca supplementation of wort may only be beneficial under certain circumstances, e.g., when wort Ca concentration is limited by the Ca con-

tent of the local water supply. Ca supplementation of mash or sparge water (100–200 ppm) has a buffering effect, which prevents a rise in the pH of wort[205]. The maintenance of a consistently low pH reduces the extraction of polyphenolic compounds and silica that may affect beer quality. The buffering effect of Ca on wort may also carry over to beer and positively influence beer characteristics such as haze formation and foam stability[205], though naturally the potential negative effects on yeast growth and fermentation potential[39,172,174] must be taken into account.

Regardless of its influence on fermentation performance or growth, Ca has been recognised for some time to play a critical role in cell flocculation[64] and the so called 'calcium bridging' hypothesis was initially proposed as an explanation of the mechanism of yeast cell flocculation[81,144]. It was suggested that the divalent Ca cation would form a link between negatively charged sites on the surface of neighbouring cells. Flocculation can however occur with concentrations of Ca as low as around 40 ppb[208], suggesting the ionic interactions alone may not be sufficient to support this phenomenon[200] and it is now generally accepted that flocculation is governed primarily by the interaction of cell surface zymolectins with mannose residues on the surface of adjacent cells[142,143], with Ca ions having a role in stabilising these bonds[200]. There is some evidence that flocculation of bottom (lager) and top (ale) fermenting strains is different with respect to Ca induction. Dengis et al.[51,52] observed that flocculation could be induced by 200 ppm Ca in lager strains, but that Ca had no effect on the flocculation of ale strains[51,52]. This is, however, not a universal phenomenon and Stratford and Assinder[200] reported that all 28 ale strains in their study were dependent on Ca for flocculation. Ale strains, which are not dependent on Ca for flocculation, are usually (though not always) part of the relatively rare 'maltose insensitive' category of brewing yeast[51,52,155]. The relationship between the two phenomena, i.e., Ca-independent flocculation and maltose-insensitive flocculation, to date is still unclear.

It is also known that laboratory culture conditions can influence flocculation. The strains included in the Dengis et al.[51,52] study, in which ale strains flocculated in the absence of Ca, were cultivated in YE (yeast extract) broth rather than wort and the results may not represent flocculation during a brewery fermentation. Ca concentrations of laboratory media (50–500 ppm) may also not be representative of the concentrations in wort, which are commonly below 50 ppm[94]. It remains to be seen if Ca concentrations in wort can limit flocculation performance. Ca ions at micromolar levels may promote flocculation[208] and it is unlikely that total wort Ca concentrations drop to these levels. Strauss and co-workers[201] described a yeast strain with an inverse flocculation pattern, i.e., it remained flocculent until stationary phase. This property was dependent on the growth media and suggested that one possible reason was the pH-dependent availability of Ca ions. Stratford[199] showed that some ale strains flocculate only within a narrow pH range and flocculate poorly in many commonly used yeast growth media because of non-optimal pH conditions. The antagonistic effects of other ions such as Li^+, K^+, Na^+, Ba^{2+}, Mn^{2+}, Sr^{2+}, Al^{3+}, Fe^{3+}, La^{3+} and Pb^{2+} [76,113,155,198,208] must also be taken into account and while these elements may, individually, be at low concentrations, their combined effect may have a significant impact on flocculation in wort containing low Ca.

Machado et al.[131] have proposed the use of flocculent brewer's yeast as a bioremediation agent for metalliferous industrial effluent. Yeast cells were capable of removing cations of Ni, Cu and Cr from waste water via biosorption of the metals to cells and removal of cells from the system through flocculation in the presence of 160 ppm Ca[131]. That brewing yeast cells retained their Ca-induced flocculation potential in the presence of excessively high concentrations of metal ions suggests that the trace metal composition of wort is unlikely to deter normal flocculation.

LIPIDS

Wort turbidity

There is considerable evidence that the turbidity or 'cloudiness' of wort is due in no small part to the presence of malt-derived fatty acids. The fatty acid component is composed mainly of longer chain fatty acids, with palmitic (C16:0) and linoleic acids (C18:2) being present at the highest concentrations[9,107,111,120,158,196,212,219].

Clarification of wort by various means in the brewhouse results in decreased levels of fatty acids[28] and in particular the longer chain and unsaturated fatty acids (UFA)[110]. The relationship between cloudiness and long chain fatty acids is not confined to brewery worts, and has also been found in wine musts[5]. Though not strictly supplements, malt derived fatty acids can be altered through modification of process parameters, or through the use of a specific brewery apparatus, e.g., lauter tun vs. filter[110].

Wort turbidity and fermentation

There has been much debate about the relative advantages of using turbid (lipid-rich) worts or bright (lipid-depleted) worts, with some commentators recommending the use of turbid or trub-rich worts (trub being a precipitation product produced during wort boiling) for their positive influence on fermentation[34,111,184,186,188,196,209], while others have advised against its use, primarily due to the potential negative effects on flavour profile and stability[156,158]. Numerous reports have shown improved fermentation performance with worts containing high levels of malt-derived solids due to improved yeast growth and viability[111,184,188], faster fermentation[111,122,184,209,221], increased uptake of wort nitrogen[196] and higher ethanol yields[121,184]. Conversely, one study has associated high gravity wort turbidity with reduced yeast growth, lower rates of attenuation and reduced uptake of FAN at both a laboratory and industrial scale[156].

It has been suggested that the use of cloudy wort improves yeast fermentation performance, not through any nutritional effect of the lipids but, rather, through solid particles acting as nucleation sites for CO_2 bubble formation[121,188]. The rationale is that the resultant wort contains less dissolved CO_2, which would otherwise have an inhibitory effect on yeast performance. However, Stewart and Martin[196] found that the use of turbid wort improved fermentation performance over and above that seen in

clear wort containing diatomaceous earth as a CO_2 nucleation factor, despite the reductions in dissolved CO_2 being identical in both cases. These results have been confirmed by Kühbeck et al.[112] who noted an increase in fermentation performance in the presence of trub, which could not be matched by the addition of other particles, including PVPP, kieselguhr and activated carbon. Improved fermentation performance in trub-rich wort may also be influenced by the presence of bound ionic metals such as Cu or Zn, which otherwise may be lost through clarification[108,111,121,184,188].

The principal objection to the use of turbid worts for fermentation relates to potential flavour effects. One of the main effects of malt-derived wort lipids on flavour profile is the effect on ester synthesis by the yeast cells, with reductions observed in the majority of studies[111,121,158,184,196]. A probable reason for this reduction in ester synthesis may be repression of the *ATF* genes[7,72,73,133,213], which encode alcohol acetyltransferases and have a major role in ester synthesis[218]. Esters typically impart a fruity or floral aroma to beers and whether a reduction in concentration is deemed desirable or not depends on the specifications of the particular beer. Indeed, Booer et al.[26] have suggested that lipid supplements derived from spent grains may be useful in removing undesirable estery notes in beer. Overproduction of esters typically occurs during high gravity fermentation[7,140]. Wort lipid modification through, for example, trub addition may have application in decreasing the disproportionate production of esters. Ester synthesis is known to be reduced by excess oxygen[7] and solubility of oxygen is reduced at higher gravities[14]. These facts may, in part, explain the higher production of esters during high gravity brewing. Lipids in fermentation wort may also have a more direct effect by imparting caprylic (soapy) notes to the flavour/aroma profile[42,227], though this characteristic is associated with medium chain-length fatty acids and may in fact be ameliorated through the addition of longer chain fatty acids to the wort or by wort oxygenation[136]. Shorter chain fatty acids may accumulate during fermentation due to the increased permeability of the yeast cell wall and contribute to stale or 'yeasty' characters in beer[137,206]. Such increased membrane permeability may arise as a result of the greater stresses to which the yeast cell is exposed during HG and VHG fermentations (discussed in further detail below). The formation of vicinal diketones (VDK) is known to correlate with a number of factors, including the presence of trub, which promote faster yeast growth and nitrogen uptake during fermentation[152]. This is, however, by no means guaranteed and Lentini et al.[121] observed no change in the concentration of diacetyl (2,3 butanedione), while Schisler et al.[184] noted a decrease in the total VDK levels in trub-rich wort. Other effects of malt-derived fatty acids are increases in the concentration of higher alcohols including iso-butanol[184,196] and propanol, 2-methylbutanol and 3-methylbutanol[196], while lower acetaldehyde concentrations have been recorded[111,184]. The presence of fatty acids in beer is typically associated with decreased flavour stability[158] and it has been suggested that the staling factor 2-trans-nonenal, which gives a distinctive cardboard taste to beer at very low concentrations[141], may arise through the oxidation of linoleic acid in beer[61,62,107].

Once again, the findings of studies investigating the relationship between lipid availability and flavour development are not unequivocal and some investigations have also found that the use of turbid wort offers no disadvantages in terms of flavour profile and stability in comparison to clear worts, and beers produced by the fermentation of very clear worts have actually rated poorly in taste tests[111,186]. Likewise, Kühbeck et al.[111] found that foam stability of the beers produced at pilot scale with turbid wort was not different to those produced from clear wort.

A number of factors must be considered before a brewer should choose a more or less clear wort. Turbid lautering may, for example, reduce the time taken to produce wort for fermentation[111], but may require more time to be spent on filtration and on cleaning of pipe lines and filters post fermentation. It is likely that optimum lipid levels exist for individual fermentation processes and yeast strains and these need to be determined empirically[2]. An interesting experimental approach might be the collection of trub after wort boiling and enzymatic fractionation and solubilisation of this trub before reintroduction of the individual fractions to the fermentation wort. This would, firstly, allow for the assessment of the effect of trub on fermentation performance without the effect of solid particle addition needing to be considered. Secondly, the effect of the respective components (e.g., UFA, sterol, bound ions etc.) could be determined.

Fatty acid supplements

While wort suspended solids are a heterogeneous collection of various compounds[153], numerous studies have highlighted the importance of individual lipid fractions, particularly palmitic (16:0) and linoleic acid (18:2), in terms of contribution to fermentation and characteristics of the beer. Linoleic acid in particular has been shown to have a significant effect on fermentation performance and beer quality. Early studies demonstrated the negative influence of pure linoleic acid on the synthesis of acetate esters by yeast[212,213]. Lentini and co-workers[121] found a direct correlation between levels of malt-derived linoleic acid in yeast cell membranes and the level of ester synthesis during fermentation. Lower concentrations of ethyl and isoamyl acetates as a direct consequence of supplementation of fermentation worts with linoleic acid have been reported in studies by Moonjai et al.[148,149]. Other effects of linoleic acid, which parallel those observed with turbid or trub-rich worts include faster fermentation rates, improved yeast growth and viability and increased levels of ethanol, but not higher alcohols[33,148,149]. Oleic acid (18:1) enrichment of wort can result in increased yeast growth and reduced ester synthesis during very high gravity (20°P) brewing[7]. Kalmokoff and Ingledew[105] reported enhanced fermentation of 30°P corn adjunct wort with a number of yeast strains when oleic acid (containing ergosterol) was added as a supplement (9.1 mL L^{1}). Supplemented wort supported greater yeast growth, alcohol levels up to 50% higher than in unsupplemented wort, and FAN utilization double that of the unsupplemented medium[105]. The addition of palmitoleic and oleic acids to fermentation wort has also been shown to result in a significant increase in nitrogen uptake by the fermenting yeast[196] and in wine must has been found to result in

greater yeast growth and fermentation performance and cause a general increase in volatile compounds[138]. The increase in ester synthesis in that particular study may have been due to increased yeast growth overriding the inhibitory effects of oleic acid[138].

The focus of studies by Moonjai et al.[148,149] has been the potential of lipid supplements to decrease the oxygen demand of the system. A smaller input of oxygen to wort will increase the flavour stability of the final beer and will limit potential oxidative stress in the yeast. An exogenous supply of UFA, which is normally synthesised via oxygen-consuming reactions, reduces the cell's demand for oxygen. The potential for UFA-supplementation to replace wort oxygenation at an industrial scale has been shown by Hull et al.[90] In this case, exposure of pitching yeast to olive oil (a source of oleic acid) prior to fermentation was used rather than wort aeration, without major effects on the acceptability of the beer produced. UFA supplementation therefore acts as a form of oxygen credit and may be of particular benefit in the case of HG and VHG gravity worts in which solubility of oxygen may be limited[14]. High gravity worts typically require a higher rate of oxygenation to ensure that the fermentation proceeds adequately, though how much of this oxygen is available for yeast metabolism and how much is lost from the wort is open to question[27]. UFA-supplementation therefore has the potential to improve fermentation performance at very high gravity, though this has yet to be determined. Increasing the unsaturation index of cellular membranes through addition of UFAs may also benefit high gravity fermentation by improving the tolerance of the yeast strain to ethanol[177], thereby allowing the fermentation to continue unimpeded.

The perception of beer quality is known to be influenced by the foaming ability of the beer, the stability of the foam and its tendency to adhere to the glass (lacing)[15,114,159]. The production of foam is initiated by the formation of bubbles at nucleation sites in the beer or on the inner surface of the glass. These bubbles are surrounded by proteins with both hydrophilic and lipophilic properties, which act to separate the hydrophobic bubbles from the beer. The interaction of the bubbles and associated proteins is known to be affected by the presence of lipids, which can bind to the foam-supporting proteins at their lipophilic sites[16,79].

Both foam retention[175] and lacing[17] have been found to be influenced by longer chain fatty acids, with shorter chain fatty acids (C_{10} and shorter) having little effect. Concentrations of the individual long chain fatty acids in wort are invariably too low to influence foam in a significant way[83,101]. However, the combined action of several long chain fatty acids could conceivably have an observable effect. It has also been found that if foam negative lipids are introduced to wort, their effect is temporally limited and, given enough time, the detrimental effect of these lipids will be negated, probably via interaction with lipid-binding proteins[17,175]. The use of immobilized lipid-binding protein has been shown to improve the foam stability of a number of commercial beers[58] and the different susceptibilities of different beers to the effects of lipids on foam[18] may be related to the relative content of lipid-binding proteins in the respective beers. Lipid-binding proteins derived from wheat have been found to improve the foam stability of beers[43,228] and this may, to an extent, explain the superior foaming characteristics of wheat beers relative to all-malt lager beers. It may be the case that other foam-negative factors introduced at the point of dispense (e.g., detergents) or during drinking (e.g., cosmetics or foods) will have a greater influence on foam characteristics than either malt- or yeast-derived fatty acids[17].

Lipids may in fact have a positive effect on foam character. For example, basic amino acids have been identified as foam negative factors by Furukubo et al.[74], a result which the authors ascribed to a possible interaction between the amino acids and foam positive iso-α-acids. It would be expected that this negative effect would be mitigated through the increased amino nitrogen uptake associated with turbid or UFA-supplemented wort[196]. The high ethanol concentrations that arise during high gravity brewing may have a negative impact on the foam-producing ability of the beer[30], though whether this effect persists after adjustment of beers to sales strength is not known. High ethanol concentrations may indirectly decrease foam stability by causing release of proteinase A during fermentation[29]. This enzyme may persist in the beer and have a negative effect on foaming potential by interfering with foam-stabilizing proteins[29]. Further research is required to determine the exact relationships between higher gravity brewing, foam character and the fatty acid content of the system. The influence of malt-derived lipids on foam may in some cases be negative[124,186] and in others neutral[111].

Effect of ethanol on the yeast cell membrane

A major factor limiting ethanol production during HG fermentation is ethanol itself. Under HG conditions ethanol levels may be as high as 16% (v/v)[36,37]. The effect such ethanol concentrations have on a cell's fermentative ability is dependent on that cell's ethanol tolerance, which is in turn affected by a number of factors including the genetic makeup of the yeast strain, the physiological state of the cells and the physico-chemical nature of the fermentation environment[27]. While the traditional view was that ethanol tolerance in yeast strains was determined by genetic differences between strains, with, for example, sake and distilling strains being more tolerant than brewing strains[177], studies have identified yeast physiology and the cell's environment as being critical to ethanol tolerance; with brewing strains in some cases tolerating concentrations similar to those experienced by sake strains[37,38,82].

The ability to produce high concentrations of ethanol without permanently damaging yeast cells and compromising fermentation performance is particularly important in brewing, as yeast populations are normally required to ferment multiple brews in succession[192]. While ethanol toxicity has been ascribed to non-specific effects on the cell[48,103], the weight of evidence suggests that membranes are the main targets of ethanol toxicity. It has been suggested that the effect of ethanol on membranes is due to its insertion into the hydrophobic interior and the resultant effects on polarity, exchange of polar molecules and the position of membrane proteins[91]. Direct ethanol exposure results in increased fluidity of membranes[103,127,145] and

increased membrane permeability causing leakage of amino acids, leakage of compounds absorbing light at 260 nm and leakage of proteases[75,91,146,182] and influx of protons[99,117,120]. The cellular membrane is not, however, the only target for ethanol toxicity and the mitochondrial membrane may also suffer damage, leading directly or indirectly to mitochondrial DNA damage and the generation of respiratory deficient (petite) strains[40,75,99]. Indeed, serial repitching of yeast in breweries has been associated with an increase in the frequency of petite mutants, probably caused by repeated exposure to potentially toxic levels of ethanol during fermentation and storage[98].

Curiously, a genome-wide study of the transcriptional responses to short-term, sudden ethanol exposure revealed significant changes in the transcription of genes involved in the general stress response, trehalose synthesis and the antioxidant response, but did not reveal any significant changes in genes directly involved in lipid membrane synthesis[4]. The reason for this is unclear and further investigation is clearly required. It may be that the stress response genes have a role in protecting cells against sudden toxic shock, while the more gradual increases in ethanol concentration that occur during fermentation elicit a response from genes involved in membrane synthesis and alteration.

Membrane fatty acid composition and stress tolerance

Sudden, acute exposure of yeast cells to ethanol is unlikely to occur either in nature or during brewery handling. It is more likely that yeast cells will be exposed to progressively higher concentrations over a period of time, with the potential for physiological adaptation to occur. Indeed, yeast cells which have been pre-exposed to ethanol often show a higher degree of tolerance than those which have not been exposed[99]. As might be expected considering the cellular targets of ethanol, the yeast cell increases its tolerance by altering membrane properties. Membrane-specific responses to ethanol include an increase in the unsaturation index and a proportional increase in longer chain fatty acids[3,19,127,157,178,181]. It is believed that these longer chain fatty acids counteract the fluidizing effect of ethanol and mitigate any potential damage that would otherwise occur with more fluid membranes[3,178]. These characteristics may be inherent or may be induced in response to ethanol. For example, Chi and Arneborg[40] found that a strain with an inherently higher membrane unsaturation index and with a high C18:C16 ratio had a relatively high tolerance to ethanol exposure (18% v/v). Aguilera et al.[1] found a relationship between ethanol tolerance and fatty acid composition of membranes (after four hours of exposure to ethanol) of a number of different yeast species. Alexandre et al.[3] found that growth in medium containing 10% ethanol led to an increase in fatty acid unsaturation, with a proportional increase in the levels of palmitoleic and oleic acids. As discussed earlier, the addition of UFA has the potential to significantly improve fermentation performance[148,196]. Thomas et al.[211] demonstrated the ability of linoleic acid addition to protect cells exposed to 5.8% ethanol. In that study, cells with membranes enriched in linoleic acid had a greater ethanol tolerance than those enriched with oleic acid. Adding linoleic acid to pitching yeast led to an increased fermentation rate in a synthetic growth medium containing 8% glucose, a result which may be, at least in part, attributable to a reduced sensitivity of the cells to ethanol. Contrary to the study by Thomas et al.[211], an investigation by You et al.[232] demonstrated that ethanol tolerance was improved most notably when the oleic acid component of the membrane was increased via medium supplementation or over-expression of the *OLE1* membrane desaturase gene. It is likely that improved ethanol tolerance contributed to the improved wort fermentation performance of yeast cells supplemented with oleic acid observed by Casey et al.[36] and Stewart and Martin[196] on a small scale, and Hull[90] on an industrial scale (with oleic acid supplied in the form of olive oil). The extent to which such additions contribute to fermentation performance through reduced ethanol sensitivity of individual cells rather than nutritional improvement and consequent increases in cell number during high and very high gravity fermentation remains to be seen.

While not the focus of this review, it is important to note that a number of process parameters such as fermentation temperature, pitching rate and oxygenation may need to be adjusted to facilitate higher gravity brewing[193]. Of particular importance is oxygenation of pitching wort to compensate for reduced O_2 solubility at higher gravity[14], especially when coupled with a higher fermentation temperature[60]. Despite its potential to cause oxidative stress, there has been no direct evidence of oxidative damage to yeast cell components under brewing conditions and oxygen's role in relation to yeast stress may rather be a positive one. As described previously, membrane composition plays a critical role in determining the cell's sensitivity to stress, and the features of aerobically grown cells (fatty acid unsaturation and increased ergosterol) are certain to contribute to the tolerance of the cells to a number of stresses, including those associated with higher gravity brewing[87].

Munoz and Ingledew[150] found that yeast cell wall material produced as a by-product of yeast extract preparation could be added to wine fermentations to overcome the phenomenon of stuck and sluggish fermentation[150]. This effect was attributed to the incorporation of longer chain fatty acids into the yeast membranes and a greater degree of unsaturation and consequent increase in ethanol tolerance. Bioethanol fermentation studies have found that the addition of lipid-rich materials such as soya flour or horse gram (*Dolichos biflorus*) flour can significantly improve the fermentation performance and viability of cells during HG bioethanol fermentations[13,125,171,210,231]. While not necessarily applicable to brewery wort fermentations, such approaches demonstrate the potential of lipid supplements for improvement of ethanol tolerance and hence fermentation performance of brewing yeast under higher gravity conditions.

While a number of studies have focussed on the impact of ethanol on the plasma membrane, less is known about the effect of hyperosmotic conditions. This, despite the fact that osmotic stress, like ethanol stress, is known to alter the fluidity of membranes[20]. Laroche and co-workers[116] reported increased tolerance to osmotic shock in cells which had been exposed to cooling prior to expo-

sure. It was hypothesised that the change in the physical state of the membrane associated with cooling reduced the effect of osmotic stress on the membrane. In another study increased membrane fluidity, achieved by the expression of sunflower desaturase genes in yeast, rendered cells less sensitive to salt (NaCl) stress, though in this study no ameliorative effect was seen with sorbitol stress[176]. It remains to be seen how membrane fluidity changes temporally during brewery handling and how different environmental parameters, such as osmotic stress (causing membrane rigidification) and ethanol stress (causing fluidization), interact to affect the physical state of the membrane during high gravity brewing and the consequent effects on stress tolerance. Furthermore, little is known about the impact of temperature on membrane fluidity, ethanol tolerance and productivity during high gravity brewing. It is known that productivity of high gravity fermentations is improved at a higher temperature[60] and that ethanol toxicity is increased at higher temperatures due to the synergistic effects of ethanol and heat on membranes[164], but how these parameters interact during fermentation requires more in-depth investigation.

Sterols

Sterols in yeast membranes influence multiple biological processes[49], but, like UFAs, may have a particularly important role in mitigating the effects of stress on cells during higher gravity brewery fermentations. While sterol levels are generally lowered when yeast cells are exposed to ethanol, increases in ergosterol concentrations have been observed[10,178]. Higher concentrations of membrane ergosterol have been found to correlate positively with ethanol tolerance[1,40,50,118,202,211]. It is possible that the protective effect of ergosterol is due to its role in reducing membrane fluidity[178,235]. Greater incorporation of ergosterol into membranes may therefore act to protect cells in the same manner as long chain fatty acids (i.e., by counteracting the fluidizing effect of ethanol). Relatively high levels of membrane ergosterol typically coincide with high unsaturation indices and greater proportions of C18 compared to C16 fatty acids[1,10,40,211] and it may be the case that both membrane components act in a concerted way to maintain optimal fluidity levels. The addition of ergosterol to a very high gravity ethanol fermentation (35% glucose w/v) was found to have no effect on fermentation performance, yeast growth or cell viability[210] when oxygen was present, resulting in sterol-replete membranes. Ergosterol cannot be synthesised in the absence of oxygen[8] and under anaerobic conditions it is taken up from the environment by facilitated diffusion[154]. The supplementation of anaerobic high gravity wort with ergosterol increased fermentation rates, productivity, uptake of amino nitrogen, cell growth and improved viability[36,60]. Furthermore, this supplementation allowed the yeast to be repitched up to five times without any appreciable change in fermentation performance[38]. Ergosterol supplements were also found to compensate for the lack of oxygen in anaerobic wort[36]. It should be noted that ergosterol is often added along with Tween-80, which acts as an emulsification agent, but is also a source of oleic acid, which may confound interpretation of results. The use of other solvents such as tergitol in experiments, for example, may clarify the role of exogenous ergosterol in protecting cells against stresses associated with higher gravity fermentation[70].

Supplementation of growth media with various sterols led to increased tolerance of yeast cells to hyperosmotic stress and this effect was most pronounced with ergosterol and the phytosterol stigmasterol[87]. In addition, increases in membrane ergosterol have also been observed in yeast cells (*Zygosaccharomyces rouxii*) exposed to salt stress[86] and there is therefore the possibility that this membrane component may also have a role in protecting cells from osmotic stress under higher gravity brewing conditions.

COMMERCIAL 'YEAST FOODS' AND YEAST EXTRACT

Yeast extract is a water soluble autolysate derived from spent yeast and contains many of the nutrients required to maintain yeast metabolism. The nutritional value of yeast extract and the abundance of spent yeast available to the brewing industry make this a potentially valuable supplement for improving the nutritional status of higher gravity worts. Yeast extract, in particular, is a rich source of freely assimilable nitrogen and may be used to balance the high C:N ratio that occurs as a consequence of adjunct brewing. A number of commercial preparations termed 'yeast foods' are available to improve the nutrient composition of wort. Yeast extract is typically a principal component of such preparations along with inorganic nutrients such as Mg and Zn[92]. Yeast foods can improve yeast fermentation performance in traditional and higher gravity worts[92,124], but have not been universally adopted by brewers. This may relate to the often undefined nature of the products. An investigation by Ingledew et al.[92] revealed that the chemical composition and nutritive value of yeast foods on the market at the time was highly variable. In many cases the products comprised high levels of non-utilizable protein and often low or undetectable levels of amino nitrogen. It was recommended that before using such a product, the brewer should be aware of its composition, the manufacturer's intended use, and any potential unwarranted or unwanted effects on fermentation performance or beer quality.

Yeast food/extract and fermentation performance

Improved fermentation performance of brewing yeast has been demonstrated in media supplemented with yeast extracts or yeast extract-based yeast foods. Such media have included glucose-rich media[13,194], *Agave azul* juice for tequila fermentation[57], as well as traditional[124], high gravity[60,139] and very high gravity[36,92] brewery worts. Benefits of yeast extract use include greater yeast growth and higher viability[36,57,109,124], shorter times to attenuation[36,57,92,109,124,139], more complete sugar consumption[13,57,109,194], and greater ethanol production[13,57,60,194]. Use of yeast extract or yeast foods may not be beneficial in all cases and careful consideration must be given to the nutritional deficiencies of a given wort and the potential of the supplement to correct these deficiencies. For example, Le Van et al.[124] found that an 11°P rice adjunct wort, contain-

ing low N levels, produced 30 g L^{-1} ethanol after 200 hours of fermentation with an ale strain. The same level of ethanol was achieved at around 100 hours when yeast extract was included. However, McCaig et al.[139] found that the ability of yeast extract to boost higher gravity (18–24°P) wort fermentation was limited by a relatively high level of FAN (approx. 270 mg L^{-1}) in the original wort. Likewise, Casey et al.[36] noted that the benefits of yeast extract supplementation were confined to adjunct worts; no improvement in fermentation performance occurred when yeast extract supplementation was increased in all-malt VHG wort. The amino acid composition, and not just FAN concentration, must be considered before a yeast food is employed. Different amino acids have different effects on fermentation performance. Proline, for example, is not typically metabolized by yeast during a brewery fermentation, but may have a role in protecting yeast against the high ethanol or osmotic stresses encountered during a sake fermentation[204] or a wheat mash fermentation for biofuel generation[210]. Thomas et al.[210] noted that wheat mash fermentations could be improved by the addition of glutamic acid (3 g L^{-1}) but not glycine. Supplementation of low N wheat mashes with lysine reduced cell viability and fermentation performance compared to control fermentations and the authors stressed the importance of the 'right' amino acids being available to fermenting yeast. Complex nitrogen sources (casamino acids, peptides) may generally be regarded as being more beneficial for fermentation performance than simple nitrogen sources such as ammonium sulphate.

Improved fermentation performance with yeast foods is, as noted by Ingledew et al.[92], not solely a result of increased nitrogen availability, and yeast foods can, in addition, contribute vitamins and metals to the fermentation. A number of studies have shown that Mg within yeast extracts or foods can contribute significantly to the fermentation performance of yeast[47,48,59]. The implication being that addition of Mg alone may, in some cases, be more advisable than adding a complex yeast food. One study attributed the beneficial effects of yeast food addition to a reduction in wort dissolved CO_2[109]. In recent years there has been a move towards development of yeast foods to correct specific wort deficiencies. Zn deficiency has, for example, been considered in the formulation of a supplement consisting of Zn-enriched intact yeast cells. These enriched cells can enhance fermentation performance over and above the effects seen with Zn salts alone[69] or other Zn-containing yeast foods[217]. This approach allows for Zn-supplementation of worts where legislation restricts the use of all but the basic ingredients in the production of beer.

Yeast foods and beer quality

The brewer's decision to include a yeast food in wort will depend not only on the influence this supplement has on fermentation characteristics, but also on the flavour profile of the resultant beer. Any gains in fermentation should not be at the expense of product quality. In this regard, the literature concerning the influence of yeast extracts/foods is limited to a small number of studies.

The presence of yeast food in standard gravity fermentation wort caused an increase in higher alcohol and ester synthesis with all eight yeast strains tested[109]. Total higher alcohol production was in some cases doubled in the presence of yeast food. This result was attributed to an improvement in the uptake of amino acids from the wort[109]. Conversely, Casey et al.[35] reported lower concentrations of most higher alcohols in beers tested when VHG wort fermentations contained yeast extract. Under the same conditions a general relative increase in ester concentration was observed, with ethyl acetate and isoamyl acetate increasing by 40% and 80%, respectively. Improved fermentation performance due to the presence of a protein-based yeast food has been found to coincide with lower acetaldehyde concentrations in beers[109]. This result was observed with all eight strains tested, though the reduction was strain-dependent and varied between 10 and 70%[109].

Evidence indicates that yeast extract supplementation may be particularly beneficial for worts containing adjuncts. Beer produced from a 40% rice adjunct wort rated poorly when evaluated by a taste panel, with diacetyl notes, wateriness and lack of mouthfeel cited as reasons for the poor evaluation. Beer produced from the same wort supplemented with yeast extract showed a significant improvement and was deemed comparable to an all-malt beer[124]. Contrary to this, McCaig et al.[139] noted that the presence of yeast food in fermenting worts (18–24°P) resulted in beers which were considered less palatable than those without supplementation. This result was most likely influenced by the lack of improvement in fermentation performance with these supplemented fermentations, relative to non-supplemented fermentations. The use of yeast extract appears to reduce the levels of VDK in finished beers. Le Van et al.[124] reported that the VDK levels of the beer produced from a 40% rice adjunct wort could be reduced from 0.26 mg L^{-1} to 0.14 mg L^{-1} when yeast extract was introduced to the wort, which was similar to levels found in all malt beers[124]. McCaig et al.[139] also reported that where yeast foods stimulated fermentation performance, there was an increase in the levels of VDK in the undiluted beers.

The compositional complexity of yeast extracts and yeast foods used to augment fermentation make interpretation of volatile and organoleptic profiles difficult. Flavour characteristics may be dependent, to a considerable extent, on the amino acid content of the supplement. Amino acid profiles of yeast extract based foods are known to vary and it is likely that this variability influences the production of esters, higher alcohols and SO_2[218]. In particular, increased availability of the branched chain amino acids is expected to lead to increased generation of higher alcohols with valine, leucine and isoleucine influencing the formation, respectively, of isobutanol, isoamyl alcohol and amyl alcohol[12,66,180,185] and to some extent also the corresponding esters[66]. It is also likely that VDK generation is influenced by the amino acid composition of yeast extracts. VDK levels are known to be influenced by FAN concentration, with valine in particular being cited as an important factor due to its influence on production of the diacetyl precursor α-acetolactate[167]. Low initial levels of valine in wort stimulate the anabolic production of valine and a corresponding increase in α-acetolactate[161]. The result is a second diacetyl peak forming during fermentation, leading to a relatively high concentration of

diacetyl in the final beer[161]. Increased wort valine added, for example, with a valine-rich yeast food, would be expected to eliminate this secondary diacetyl peak and result in beers which require less time to mature. Other amino acids may have a less direct effect on VDK level. For example, Lekkas and co-workers[119] found reduced VDK levels with methionine supplementation and increased levels with lysine supplementation. In this case the differences in VDK concentration were due to the longer and shorter fermentation times for methionine-supplemented (103 hours) and lysine-supplemented (48 hours) worts when compared to control wort fermentations (96 hours)[119]. Amino acids may also have a direct effect on beer flavour with proline, for example, imparting a sweet taste[204]. Hydrogen sulphide generation during wort and must fermentation is known to increase with increased availability of the sulphur-containing amino acid cysteine[63,100]. Duan et al.[63] measured H_2S production by nine yeast strains (six lager and three ale) in 14°P wort with different additions of cysteine (50, 100 or 150 ppm). In each case cysteine supplementation resulted in increases in H_2S, with concentrations increasing by between 1.7-fold and 6.7-fold depending on the strain involved. In the same study, methionine addition did not increase H_2S levels and, in fact, lowered H_2S production in cysteine-supplemented fermentations. The underlying causes of these phenomena are unclear.

CONCLUSIONS

Despite decades of research, the potential of very high gravity brewing (20°P and upwards) has, to date, not been realised. Current concerns regarding energy consumption and availability of raw materials has given a new impetus to research in this field. In this review the potential for nutritive enhancement of VHG wort for improved fermentation performance and the potential pitfalls associated with this approach have been outlined. A diverse range of supplements are available for improved VHG fermentation and in recent years our understanding has been improved by research carried out to enhance the efficiency of bioethanol fermentations. Brewery fermentations are, however, clearly more complex due to the various parameters (fermentation, flavour, foaming, yeast vitality) that need to be considered when adding supplements to wort. Supplements such as whey may improve fermentation efficiency, but otherwise be inappropriate for beer production.

A further complication is that wort is a relatively ill-defined medium and the roles of various wort components such as oligopeptides, vitamins, nucleotides, etc. are not fully understood. A more comprehensive knowledge of wort composition and the interactions between different wort components will, no doubt, assist in the choice of supplement composition and concentration required for VHG fermentation. Likewise, many potentially useful supplements such as yeast foods, are not well defined and this must be taken into consideration before they are used. A relatively simple approach would be the use of mono-component supplements, Mg salts, specific UFA, etc. to correct specific nutrient deficiencies. This approach, while having advantages in terms of simplicity, will in many cases not be feasible due to the expense of pure compounds. It is also unlikely that VHG worts will have a single nutrient deficiency. The use of complex supplements such as yeast extracts or hulls is a practical approach, but will inevitably have unforeseen and unwanted effects on fermentation performance, beer quality, etc. It would be of value to screen a number of complex supplements to see which are most effective at improving overall fermentation performance at higher gravity and then, using a step-wise approach, identify which of the individual components are involved in specific improvements in fermentation.

Results to date have shown that the response of yeast to a particular wort addition is highly strain-dependent and supplementation should ideally be tailored to specific worts and yeasts. Future research could also focus on the pairing of barley varieties with yeast strains. It may be that identification of the potential nutritive properties of specific barley varieties and nutrient requirements of specific yeast can be matched to alleviate nutrient deficiencies in wort without any additional supplements being required.

The successful implementation of VHG brewing would have significant advantages in terms of environmental sustainability and cost reduction. Success will, however, require a holistic approach combining not just nutritional augmentation of wort, but also modification of brewing parameters and careful strain selection or improvement.

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