Beer: An Ancient Yet Modern Biotechnology

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Abstract: The brewing of beer is a complex process that draws on a diversity of sciences and technology, of which chemistry is but one. This paper focuses on the chemistry of the brewing process and of the finished product. It examines each of the main classes of molecule found in beer, considers their contribution to quality and their origins in the brewing process. The study of beer and its production provides an excellent illustrative example for teaching how raw materials and the manner by which they are processed determine the acceptability of a product. Beer, whilst 90%+ water, contains a wide range of chemical species which establish its properties. Apart from ethanol (the common denominator amongst all alcoholic beverages), beer contains substances that determine its flavor, foam, and color. The flavorsome components of beer include the bitter iso-a-acids and aromatic essential oils from hops, along with esters, acids, sulfur-containing compounds and vicinal diketones from yeast. The foaminess of beer depends on the presence of carbon dioxide but also of surface-active materials like amphipathic polypeptides from malt and the bitter substances from hops. The color is due to Maillard reaction products generated largely during the kilning of malt. The malting and brewing processes (which are briefly described) are designed to maximize the extraction and digestion of barley starch and protein, yielding highly fermentable wort. The processes are also designed to eliminate materials that can have an adverse effect on beer quality, such as the haze-forming polyphenol from barley and hops and the lipids and oxygen that, together, can cause beer to stale.

Introduction

It is generally overlooked that biotechnology is not a late 20th century phenomenon. In brewing we have an established, mature industry that is perhaps mankind's oldest biotechnology. It constitutes a sophisticated application of good science, involving the controlled digestion and extraction of vegetative systems (barley and hops), to generate a feedstock (wort), which in turn is used in a highly regulated fermentation employing yeast. And, further downstream, there is a refining process of considerable complexity.

The breadth of science involved in converting barley to beer is huge—and includes botany, agronomy, plant physiology, biochemistry, chemical engineering, microbiology, physics, and sensory and consumer studies. And, of course, chemistry. The breadth of chemical species involved, from flavor-active small molecules to polymers such as starches and proteins, is formidable.

Thus, beer, and its production via the processes of malting and brewing, presents an excellent vehicle for the student to understand and appreciate how raw materials and the way in which they are processed impact on the quality of an end-product. It illustrates how food systems can be quite complex, yet how a knowledge of the chemistry allows achievement of a well-controlled process and product.

The Nature of Beer

The properties of beer are due to a wide range of chemical compounds, both large ones (macromolecules) and small ones. Relatively few chemicals are added to beer; many are derived from its raw materials. In some markets propylene glycol alginate can be used as a foam stabilizer [1] and sulfur dioxide or ascorbic acid as a flavor stabilizer [2]. Chiefly, however, the chemical constituents of beer derive either directly from the malted barley, adjuncts, water and hops, which are the raw materials of beer production, or arise through the agency of yeast, which performs the alcoholic fermentation that is at the heart of beer production. In some markets, notably Germany under the Bavarian purity law of 1516 (the *Reinheitsgebot*), only malted barley, hops, yeast, and water may be used for the production of beer.

In this paper I will examine each of the main classes of molecules found in beer and consider their contribution to quality and their origins in the brewing process.

The Basics of Malting and Brewing

Fundamentally, beer is the product of the alcoholic fermentation by yeast of extracts of malted barley (Figures 1 and 2). Whilst malt and yeast contribute substantially to the character of beers, the quality of beer is at least as much a function of the water and, especially, of the hops used in its production.

Barley starch supplies most of the sugars from which the alcohol is derived in the majority of the world's beers. Historically this is because barley, unlike other cereals such as wheat, retains its husk on threshing and this husk traditionally formed the filter bed through which the liquid extract of sugars was separated in the brewery.

The starch in barley is enclosed in cell wall and proteins, and these wrappings are stripped away in the malting process (essentially a limited germination of the barley grains), leaving the starch largely preserved. This softens the grain and makes it more readily milled, and permits removal of unpleasant grainy and astringent characters during malting.

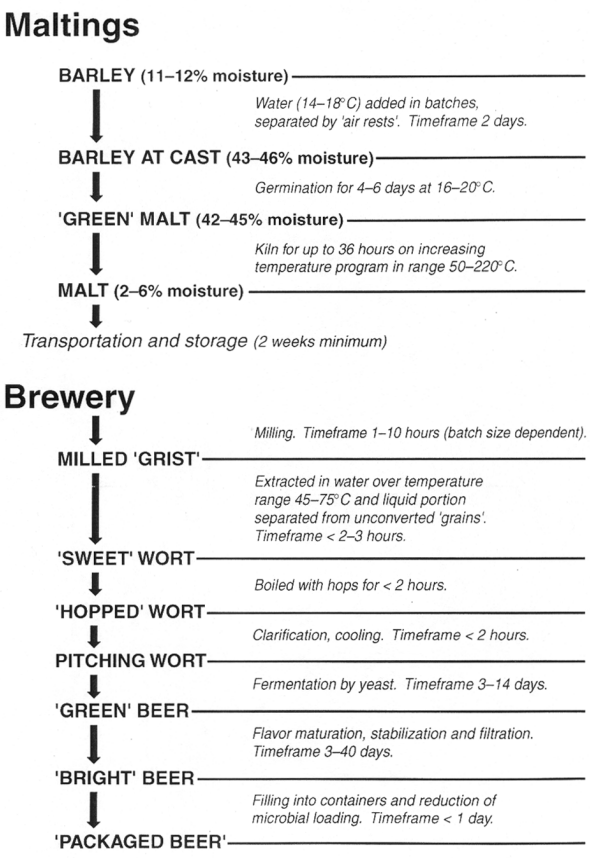

Figure 1. The key stages in malting and brewing.

Malting. Malting commences with steeping of barley in water at 14–18 °C for up to 48 h, until it reaches a moisture content of 42–46%. This is usually achieved in a 3-stage process; the steeps being interspersed with "air rests" that allow the barley to get some oxygen (to "breathe").

Raising the moisture content allows the grain to germinate, a process that usually takes 3–5 days at 16–20 °C. In germination, the enzymes break down the cell walls and some of the protein in the starchy endosperm, which is the grain's food reserve, rendering the grain friable. Amylases, produced in germination, are important for the mashing process in the brewery.

Lowering the moisture content by kilning arrests germination, and regimes with progressively increasing temperatures from 50 to perhaps 110 °C are used to allow drying to <5% moisture whilst preserving heat-sensitive enzymes. The more intense the kilning process, the darker the malt and the more roasted and burnt are its flavor characteristics. Essentially, malts used for making lager-style beers are kilned relatively gently, whereas those going into ales are subjected to more heating. The very dark colors in stouts are achieved through the use of a proportion of malt that is roasted intensely.

Brewing. In the brewery, the malted grain must first be milled to produce relatively fine particles, which are for the most part starch. The particles are then intimately mixed with hot water in a process called mashing. The water must possess the right mix of salts. For example fine ales are produced from waters with high levels of calcium. Famous pilsners are from waters with low levels of calcium. Typically, mashes have a thickness of three parts water to one part malt and contain a stand at around 65 °C, at which temperature the granules of starch are converted by gelatinization from an indigestible granular state into a "melted" form which is much more susceptible to enzymatic digestion.

The enzymes that break down the starch are amylases. They are developed during the malting process, but only start to act once the gelatinization of the starch has occurred in the mash tun (vessel). Some brewers add starch from other sources, such as maize or rice, to supplement that from malt. These other sources are called "adjuncts."

After perhaps an hour of mashing, the liquid portion of the mash, known as wort, is recovered either by straining through the residual spent grains (lautering) or by filtering through plates. The wort is run to the kettle (sometimes known as the copper, even though kettles are nowadays fabricated from stainless steel) where it is boiled, usually for one hour. Boiling serves various functions, including sterilization of wort, precipitation of proteins (which would otherwise come out of solution in the finished beer and cause cloudiness), and the driving away of unpleasant grainy characters originating in the barley. Many brewers also add some adjunct sugars at this stage, at which most brewers introduce at least a proportion of their hops.

The hops have two principal components: resins and essential oils. The resins (so-called α-acids) are changed ("isomerized") during boiling to yield iso-α-acids, which provide the bitterness to beer. This process is rather inefficient. Nowadays, hops are often extracted with liquefied carbon dioxide and the extract is either added to the kettle or extensively isomerized outside the brewery for addition to the finished beer (thereby avoiding losses due to the bitter substances' tendency to stick on to yeast).

The oils are responsible for the "hoppy nose" on beer. They are very volatile and if the hops are all added at the start of the boil then all of the aroma will be blown up the chimney. In traditional lager brewing a proportion of the hops are held back and only added towards the end of boiling, which allows the oils to remain in the wort. For obvious reasons, this process is called late hopping. In traditional ale production, a handful of hops is added to the cask at the end of the process, enabling a complex mixture of oils to give a distinctive character to such products. This is called dry hopping. Liquid carbon dioxide can be used to extract oils as well as resins; these extracts may also be added late in the process to make modifications to beer flavor.

After the precipitate produced during boiling has been removed, the hopped wort is cooled and pitched with yeast. There are many strains of brewing yeast (*Saccharomyces cerevisiae*), and brewers carefully protect their own strains because of their importance in determining brand identity. Fundamentally, brewing yeast can be divided into ale and lager strains, the former collects at the surface of the fermenting wort and the latter settles to the bottom of a fermentation (although this differentiation is becoming blurred with modern fermenters). Both types need a little oxygen to initiate their metabolism, but otherwise the alcoholic fermentation is anaerobic. Ale fermentations are usually complete within a few days at temperatures as high as 20 °C, whereas lager fermentations at as low as 6 °C can take several weeks. Fermentation is complete when the desired alcohol content has

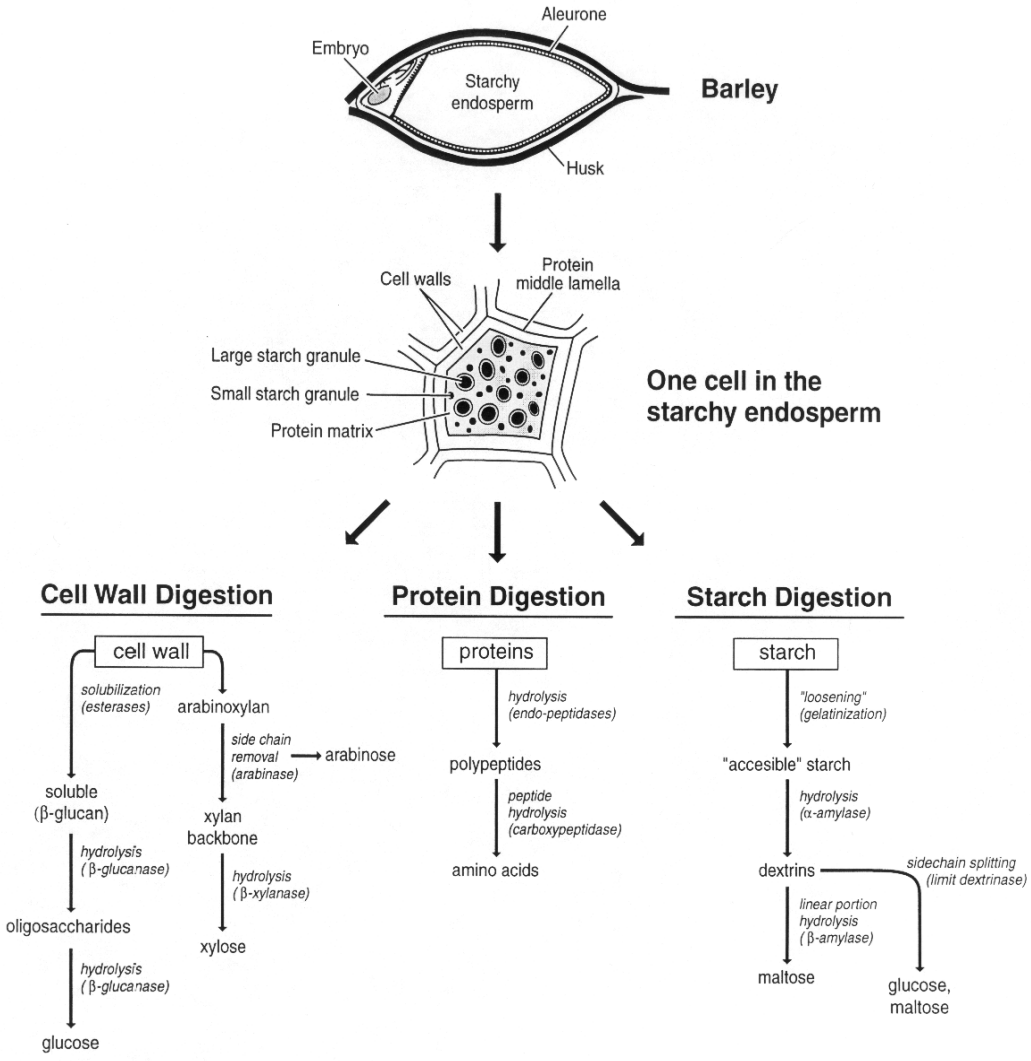

Figure 2. Barley and its digestion in malting and brewing.

been reached and when an unpleasant butterscotch flavor, which develops during all fermentations, has been mopped up by yeast. The yeast is harvested for use in the next fermentation.

In traditional ale brewing, the beer is now mixed with hops, some priming sugars, and with isinglass finings from the swim bladders of certain fish, which settle out the solids in the cask. In traditional lager brewing, the "green beer" is matured by several weeks of cold storage prior to filtering.

Nowadays, many beers, both ales and lagers, receive a relatively short conditioning period after fermentation and before filtration. This conditioning is ideally performed at –1 °C for a minimum of three days, under which conditions more proteins drop out of solution, making the beer less likely to go cloudy in the package or glass. The filtered beer is adjusted to the required carbonation before packaging into cans, kegs, or glass or plastic bottles.

It is beyond the scope of this article to describe the breadth of beer styles. Beers, however, can be broadly distinguished into two overall types: ales (including stouts) and lagers. Traditionally, ales were produced from relatively well-modified malts that had been kilned to quite high temperature regimes (very intense regimes for the darker stouts) and fermented using yeasts that rise to the surface during fermentation. The strong "dry" hop character of ales was traditionally established by adding a proportion of the hops to the finished beer. Lagers were produced from less well-modified malt, kilned relatively gently, with yeasts that sink to the bottom of the fermenter. The "late" hop character of lagers was established in the kettle by adding a proportion of the hops just before the end of boiling. In reality nowadays there has been a blurring of these features. (A more thorough and detailed description of the history and world of beer is available [3])

The Chemistry of Beer

Ethanol. Ethanol is a key component of beer, as it is of any other alcoholic beverage. Ethanol interacts with the human body in several ways [4], influencing performance and various aspects of health, both beneficially and detrimentally.

Many studies have indicated that moderate alcohol intake has a positive impact on health, in particular by protecting against cardiovascular disease [5]. The rationale for this is undoubtedly complex, but probably alcohol reduces the level

of fatty deposits on artery walls and lessens the cohesiveness of blood platelets, thereby making them less likely to "clot."

The effects of alcohol on the quality of beer are diverse. Ethanol contributes directly to flavor. Furthermore, it may moderate the contribution of other components to flavor by influencing the partitioning of those molecules between the body of the beer and its headspace.

Ethanol also influences the foaming properties of beer [6]. It lowers surface tension, and therefore promotes bubble formation. However, ethanol also competes with other surface-active molecules (notably proteins) for places in the bubble wall; this detracts from stability of the head.

It is customary to talk about beer strength in terms of alcohol by volume (ABV), in units of cubic centimeters of ethanol per 100 cm^3 of beer. Occasionally one encounters alcoholic strength described in terms of weight per volume. Bearing in mind that the specific gravity of ethanol is 0.79, this means that a beer that contains 5% alcohol by volume has approximately 4% alcohol by weight.

Compared to other alcoholic beverages, beer contains relatively low levels of ethanol. In the United States the average alcoholic strength of beer is 4.6% ABV, whereas a wine may be in the range of 9–15% ABV and spirits may be 40% ABV.

Carbon Dioxide. Carbon dioxide is, of course, produced mole for mole alongside ethanol during the fermentation of glucose by Brewers' yeast, *Saccharomyces cerevisiae*:

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2\ C_2H_5OH + 2CO_2$$

Carbon dioxide provides the "sparkle" in beer, affording a pleasurable pain sensation through interaction with the trigeminal nerve. Like ethanol, it plays a substantial role in establishing the quality of beer. Apart from its influence on "mouthfeel", CO_2 determines the extent of foamability (foam formation) and naturally influences the delivery of volatiles into the headspace of beers.

Most cans or bottles of beer contains between 2.2 and 2.8 volumes of carbon dioxide (i.e., 2.2–2.8 cm^3 CO_2 dissolved in 1 cm^3 of beer). At atmospheric pressure and 0 °C, a beer will dissolve no more than its own volume of CO_2, so achievement of these high levels of CO_2 demands the pressurizing of beer. Even so, when the cap is taken off a bottle of beer, the gas normally stays in solution and the beer is said to be "supersaturated". Foam is produced when gas bubbles form by nucleation, which occurs at scratches on beer glasses or on particles or microbubbles generated when beer is agitated, for example, by pumping, shaking, or pouring with vigor from a height [7].

Sometimes this bubble formation occurs spontaneously when a bottle or can of beer is opened—an annoying event called "gushing" [8]. It may be caused by shaking of the container (beer should be allowed to stand chilled for a little while after it has been moved around), but in extreme cases gushing can occur for other reasons such as fungal contamination of the malt used to make the beer.

Other Gases in Beer. Two more gases from air can be found in beer. Oxygen, which can enter into beer when it is transferred between tanks and during the packaging process unless precautions are taken, is severely detrimental to quality because it oxidizes components of beer, leading to staling and the formation of haze [9]. Various constituents of beer when oxidized might lead to the short chain carbonyls (e.g., *trans*-2-nonenal) which cause the papery-cardboard character associated with staleness [10]. These substrates include higher alcohols (such as 3-methylbutanol), the iso-α-acids (which provide bitterness), and the unsaturated fatty acids (see later).

The most oxidizable molecules in beer are polyphenols [11]. On one hand, this serves to protect beer against staling, as these substances act as "oxygen sinks" [12]. However, following their oxidation they polymerize and cross-link with proteins (the tanning reaction) to form insoluble complexes which afford an unsightly turbidity to beer [13]. Generally speaking, the brewer seeking to err on the side of caution removes as much polyphenol as possible by adsorbtion onto polyvinylpolypyrrolidone after the filter. Modern packaging techniques facilitate oxygen levels as low as 0.1 mg/L in fresh beer.

Nitrogen has been added to beer for many years to promote foam stability, mostly in Ireland and the United Kingdom [14]. The foams produced by N_2, as compared to CO_2, have much smaller bubbles, which are much more stable than larger bubbles. As little as 20 mg of N_2 per liter is sufficient to enhance beer foam quality, levels which are vastly lower than those of CO_2. Nitrogen also makes the texture of a beer much smoother, but it does have a tendency to suppress the hoppy aroma that is required of some beers, and results in beers that are sometimes described as "empty" or "flat."

Water. Beer is 90–95% water, thus it is no small wonder that its composition is so critical as a determinant of beer quality. Brewing demands much more water (5–20-fold more) than that which ends up in the beer as a lot of water is needed for cleaning and for raising the steam needed for heating vessels [15].

The water must contain no taints or hazardous components, so a brewer may treat all water coming in to the brewery by procedures such as charcoal filtration and ultrafiltration [16]. The water must also have the correct balance of ions [17]. Traditionally, ale brewing was established in towns, such as Burton-on-Trent in England, where the level of calcium in the water is relatively high (ca. 350 mg/L) It is claimed that this is good for ales, whereas low levels of calcium, such as the less than 10 mg/L in Pilsen, is best for bottom-fermented lagers. A scientific justification for this is not entirely proven, but may relate to the role of calcium in promoting the surface behavior of the top-fermenting ale yeasts. In many places in the world, the salt composition of the water is adjusted to match that first used by the monks in Burton in the year 1295, a process known as "Burtonization." Often the brewer will simply add the appropriate blend of salts to achieve this specification. To match a Pilsen-type water it is usually necessary to remove existing dissolved ions by deionization, perhaps by a filtration technique.

Calcium plays several roles. It promotes the action of α-amylase, which is the first enzyme of attack on starch [18]. It reacts with phosphate from the malt to lower the pH to the appropriate level for mashing [19]:

$$3\ Ca^{2+} + 2\ HPO_4^{2-} \rightarrow Ca_3(PO_4)_2 + 2\ H^+$$

Calcium also precipitates another natural component of malt, oxalic acid, which otherwise would survive into the beer and cause problems such as haze, gushing, and the blocking of dispense pipes ("beer stone") [20] and this is the clearest justification for the Burtonization process referred to earlier. Calcium also promotes the flocculation of yeast [21].

Carbohydrates. Figure 3 briefly summarizes some aspects of carbohydrate structural chemistry relevant to malting and brewing.

Whilst the majority of the sugar found in wort is fermented to ethanol by yeast, some carbohydrates do remain in the beer. Furthermore, extra sugars ("primings") may be added to sweeten the final product [22].

The carbohydrates surviving into beer from wort are the nonfermentable dextrins and some polysaccharide materials. The dextrins are remnants of starch degradation, whereas the polysaccharides derive from cell walls in barley.

Most of the starch in the endosperm of barley survives malting because it is relatively resistant to enzymatic hydrolysis over anything other than prolonged contact times. However, if starch is gelatinized (which can be likened to melting) by heat treatment, then its constituent molecules, amylose and amylopectin, become much more accessible to the enzymes of attack [23]. Thus the start of brewing involves gelatinization, typically at 65 °C, a stage known as "conversion". Other cereals, which may be used as adjuncts, have starches that need higher gelatinization temperatures, such as rice and corn, in which starch gelatinizes over the range 70–80 °C [24]. They are cooked separately from the main mash and then mixed with the malt mash in order for the enzymes from malt to degrade them alongside the barley starch.

Enzymatic hydrolysis of starch commences with α-amylase, which is a very heat resistant enzyme, well able to survive mashing at temperatures as high as 75 °C [25]. It is an endo-acting enzyme, which means that during conversion it catalyzes the hydrolysis of α-1,4 bonds within the starch, releasing dextrins. Action of this enzyme on amylose releases linear dextrins, but the dextrins produced from amylopectin through the agency of α-amylase have side chains (they are branched).

The next enzyme involved in the starch degradation cascade is β-amylase [26]. This is an exo-enzyme, in that it approaches its substrate (the dextrins) from the outside of the molecule, chopping off two glucosyl units at a time (i.e., maltose). Beta-amylase is sufficiently heat-stable for a significant proportion of it to survive mashing at 65 °C, but at higher temperatures it is progressively more rapidly inactivated. Thus, if mashing is carried out at, say, 72 °C, then the wort produced has a high dextrin level but a low content of fermentable sugars (glucose, maltose, maltotriose). This high-temperature mashing technique is used to make low-alcohol beers [27].

The other important feature of β-amylase is that it cannot pass the branch points in the dextrins formed from amylopectin. A third enzyme, limit dextrinase, is needed to carry out this function [28]. However this enzyme is generally present in fairly low concentrations in mashes, primarily because it is developed very late during the malting process [29]; most commercial malts thus will contain relatively low levels. Additionally, the enzyme tends to be bound up with "blocking" protein [30]. Limit dextrinase is also the most heat-labile of the starch-degrading enzymes and is soon lost once the gelatinization temperature has been exceeded [31].

A range of beers is available, which are termed "superattenuated" but generally marketed as "Light," in which all of the available starch is converted into ethanol. To effect this, an exogenous heat-stable glucoamylase or pullulanase of microbial origin is added to the mash or to the fermenter [32]. The world's first approved genetically-modified brewing yeast was transformed to express a glucoamylase, however as yet this strain has not been used in any commercial operation [33].

The starch in barley is enwrapped in a matrix of protein, which is in turn enclosed by cell walls [34]. It's rather like a Snickers bar, with the wrapping equating to the cell wall, the chocolate to the protein, and the starch granules to the nuts within. To get at the contents, one has to strip away the wrapping; thus, to access the protein and then the starch, the cell walls have to be removed.

The major component of the cell walls in barley is a β-glucan comprising β 1–4 links (as in cellulose) but disrupted by occasional β 1–3 links, which makes the molecule less intractable than cellulose. It is hydrolyzed by endo-β-1,3; 1,4 -glucanase [35], an enzyme that is extremely heat-sensitive, losing all of its activity within 5 minutes of mashing at 65 °C [36]. Whilst one of the main purposes of malting is to degrade the cell walls through the action of this enzyme during germination, in practice some glucan always survives into malt [37]. Unless it is properly degraded, it renders the wort extremely viscous, with attendant problems in the operations of separating the wort from the spent grains and with downstream beer filtration [38]. Thus some brewers "mash-in" at low temperatures (perhaps 50 °C) to allow β-glucanase to act before raising the heat to conversion temperature. Additionally, a heat-stable glucanase from bacteria (such as *Bacillus subtilis*) or fungi (such as *Trichoderma reesei* or *Penicillium funiculosum*) may be employed [32]. Barley has been transformed to express a heat-resistant β-glucanase, but has not yet been cleared for commercial use [39].

Proteins, Polypeptides, and Amino Acids. The presence of polypeptide material in beer is important for the contribution it makes to foam [40]. Amphipathic polypeptides build the backbone of the bubbles in beer foam [41]; the hydrophobic regions of these molecules drive them away from the bulk beer and into the surfaces of bubbles where they interact with other hydrophobic molecules, notably the bitter substances (iso-α-acids, see later) in interactions that stabilize the foam.

Analysis of the polypeptide distribution in foams reveals a fairly heterogeneous mix of molecules, united by the common feature of hydrophobicity [42]. In the processes of malting and brewing, the native proteins of barley undergo considerable degradation and denaturation, such that those present in the finished beer bear little resemblance to those found in the barley kernel. Whilst polypeptides can be beneficial for foaming, they are detrimental in another respect: they can cross-link with polyphenols to form hazes [13].

The amino acids in beer provide no real benefit; if present in excess, they potentiate infection of a product by acting as nitrogen sources for spoilage micro-organisms. It is important that wort contains the correct balance of amino acids to support yeast growth and fermentation of that wort. Sufficient proteolysis must occur during malting and mashing to generate

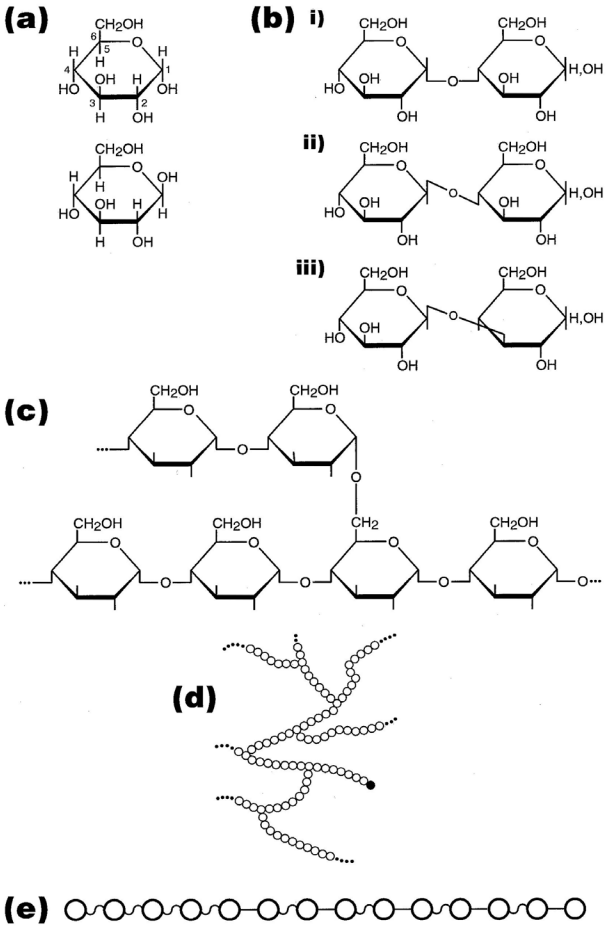

Figure 3. Carbohydrate structures. a.) Glucose in its pyranose (6 membered) ring form. The numbering convention for C atoms is shown in the top structure, which is designated α to indicate that the –OH at carbon-1 is lowermost. In the other structure (β), the –OH is uppermost at C-1. b.) (i) Maltose, wherein is an α1→4 bond between the two glucosyl residues, (ii) cellobiose, wherein is a β1→4 bond between the two glucosyl residues, (iii) laminaribiose, wherein is a β1→3 bond between the two glucosyl residues. c.) A portion of the structure of the amylopectin fraction of starch, showing an α1→6 branch point. d.) A schematic representation of amylopectin. O indicates a glucosyl residue; • indicates the single reducing end. e.) A schematic representation of part of a barley β-glucan molecule. A "~" indicates a β1→4 bond;—indicates a β1→3 bond.

these amino acids and to remove haze-potentiating proteins, whilst leaving ample foam-positive polypeptide in the malt.

During germination of barley, endo-enzymes (in the albumin fraction) called proteases [43] are developed in the grain; these are capable of attacking the hearts of protein molecules to release polypeptides and peptides (shorter chains of amino acid residues). A second enzyme, called carboxypeptidase, acts on the products of protease action during malting and mashing to release individual amino acids by splitting off one amino acid at a time from the carboxyl end of peptides [44].

Lipids. Barley contains about 3% w/w lipid [45], most of it congregated in the living tissues (embryo and aleurone).

Very little lipid, however, survives into beer, making this beverage essentially a fat-free food. This is just as well, from an aesthetic point of view, because lipids are very bad news

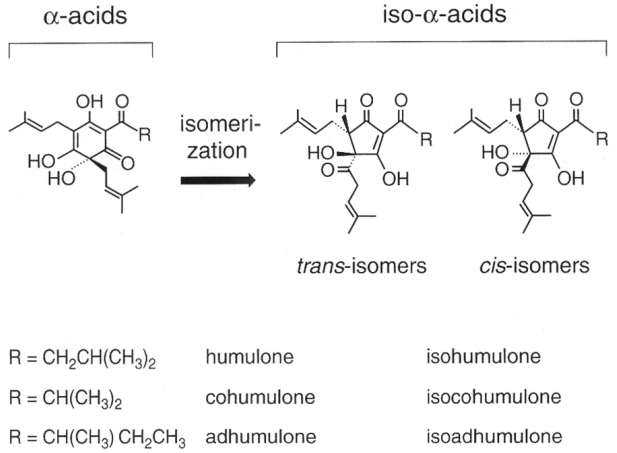

Figure 4. Bitter compounds from hops.

for beer foam [40]. Like ethanol, they disrupt the network of proteins in the bubble wall and cause the bubbles to burst. This is the reason why foam is rapidly killed if any lipid enters into beer in the glass, for instance from greasy residues clinging to the moustaches of male drinkers or the lipstick of (generally) female drinkers. Detergents behave in the same way, and for this reason beer glasses should be thoroughly rinsed with clean water after a detergent wash.

The other adverse influence of lipids is through their ability to act as precursors of stale flavors in beer [46]. The unsaturated fatty acids, such as linoleic acid, may get good press for their health-giving properties, however, they can be oxidized to ultimately yield carbonyl compounds that have undesirable flavor characteristics, such as cardboard. For this reason many brewers try to ensure that as little lipid as possible survives the brewing process and therefore they are meticulous about eliminating solid material at all stages, because the insoluble lipid associates with solids. Of much more practical significance, however, is the elimination of oxygen [9].

Flavors from Hops. Hops play several roles in the production of beer [47], but in particular they are crucial as a source of bitterness (from the hop resins) and aroma (from the essential oils).

The chemistry of hop resins is somewhat complex, but of most importance are the α-acids (Figure 4) which can account for between 2 and 15% of the dry weight of the hop, depending on variety and environment. The higher the α-acid content, the greater the bitterness potential.

There are three different α-acids in hops, which differ in their side-chain structure. When wort is boiled, the α-acids are isomerized to form iso-α-acids (Figure 4). The latter are much more soluble and bitter than the α-acids. Isomerization in a boil is not very efficient, perhaps no more than 50% of the α-acids are isomerized and less than 25% of the original bittering potential survives into the beer. Each iso-α-acid exists in two forms, cis and trans, which differ in the orientation of the side chains (Figure 4). The six iso-α-acids differ in the quality and intensity of their bitterness; it is generally felt that the better hops have a lower level of cohumulone, giving lower levels of the iso-α-acids derived from this material [48].

Apart from imparting bitterness to beer, the iso-α-acids also promote foaming by cross-linking the hydrophobic residues on polypeptides with their own hydrophobic side-chains [49],

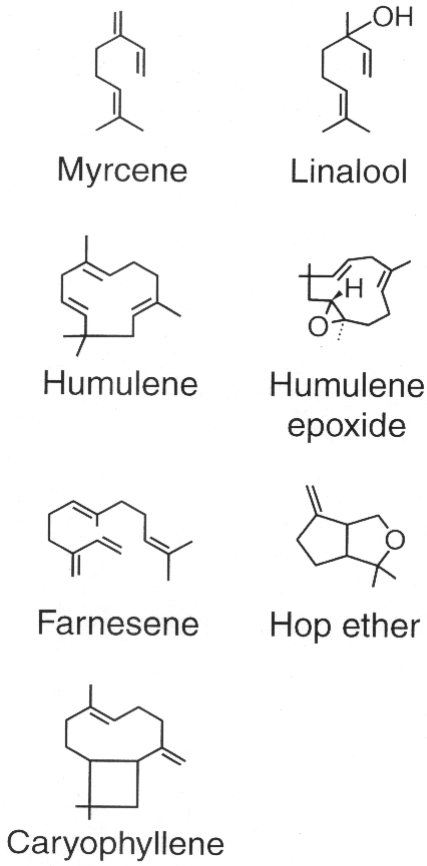

Figure 5. Some aroma compounds from hops.

rendering the foam almost solid-like and able to cling to ("lace") the walls of the drinking glass [50]. Furthermore they have strong antimicrobial properties and are able to suppress the growth of many Gram-positive bacteria [51].

Hops contain between 0.03% and 3% w/w of oil, which comprises a complex mixture of at least 300 compounds (Figure 5) [52]. As yet, there is a very incomplete understanding of the relationship between oil composition in the hop or the beer and perceived "hoppy" character. It is likely that "late hop character" (so-called because it arises from hops added late in the kettle boil, in order that excessive loss of aroma potential is not lost through volatilization) is due to the synergistic action of several oil components, perhaps modified by the action of yeast in fermentation [53]. "Dry hop character" (associated with traditional English cask ales, in which whole hops are added to the finished beer with a concomitant high level of oil "leakage" into beer) is no less complicated. The introduction of a given hop character depends on the skill of the brewer in adding the hops at exactly the right time to ensure survival of the right mix of oils that imparts a given character to the beer.

The use of whole cone hops is rare nowadays, although the largest brewing company in the world is adamant that they should still be used because their use ensures top quality in a product. More commonly hops are used after hammer-milling and extrusion into pellets, in which form they are more stable and more efficiently utilized [54]. Because of the poor utilization of α-acids in wort boiling, even from pellets, and

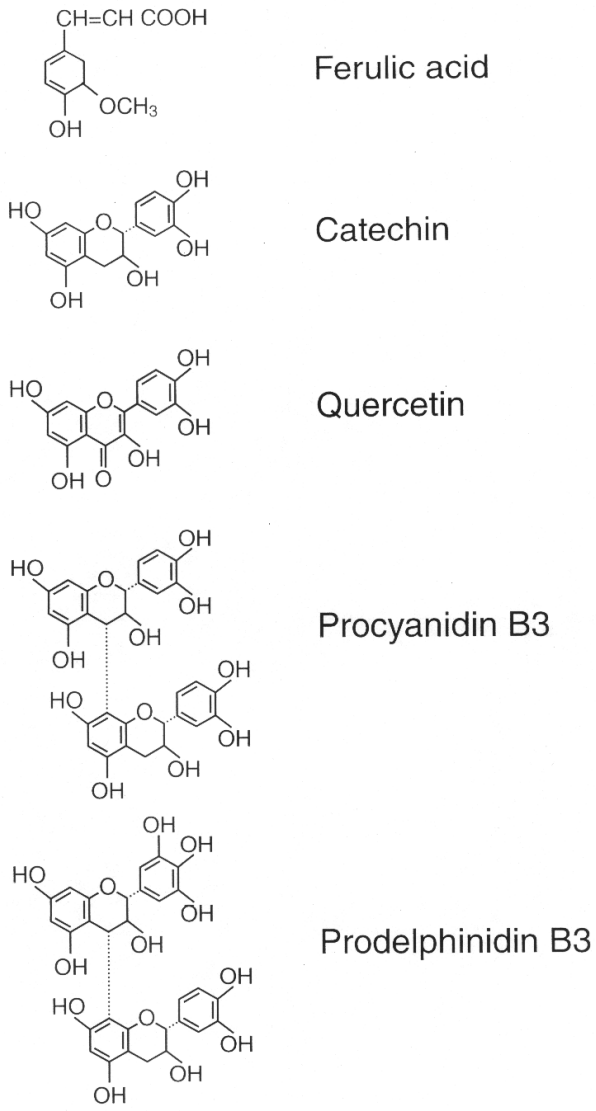

Figure 6. Some phenolic components of beer.

because of the difficulties involved in consistently introducing hoppy aromas into beers, a wide range of hop preparations have been developed [55]. These are based on liquid carbon dioxide extracts of hops; the extracted resins; isomerized by weak alkali; are ready for addition to beer downstream. The oils can be separated from the resins and also added late to create individual flavors [56].

Increasingly used nowadays are isomerized resin extracts in which one or more of the side chains of the iso-α-acids have been reduced using hydrogen gas in the presence of a palladium catalyst [57]. This is done because one of the side chains is susceptible to cleavage by light, yielding a radical breakdown product that reacts with traces of sulfidic materials in beer to produce 3-methyl-2-butene-1-thiol (MBT), a compound which affords a disgusting, skunky aroma [58]. If the side chain is reduced, it no longer produces MBT. For this reason, beers that are likely to be exposed to light in package (e.g., by being sold in green or clear glass bottles) often contain these modified bitterness preparations, which have the

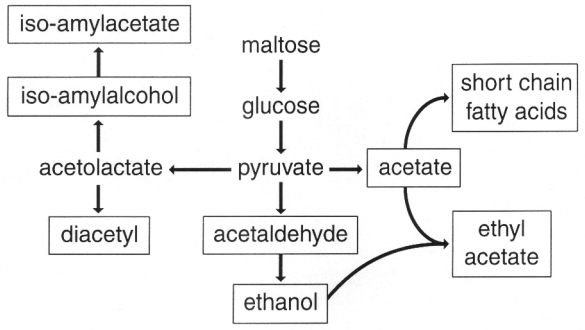

Figure 7. Pyruvate as the start point for flavor compound production by yeast.

added advantage of possessing increased foam-stabilizing properties.

Phenolic Materials. In just the same way that the chemistry of the essential oil fraction of hops is enormously complex, so too is that of the phenolic materials contributed to beer by both barley and hops [59] (Figure 6).

Perhaps the simplest such material of significance to beer quality is ferulic acid, a molecule found in the endosperm cell walls of the starchy endosperm. It is in theory at least, though perhaps not in practice, able to act as an antioxidant [12]. It is also prone to decarboxylation, a reaction catalyzed by an enzyme present in various microorganisms, but happily not in strains of brewing yeast [60]. The product of this reaction, 4-vinylguaiacol (4-VG), has an intense flavor of cloves and, as a result, contamination of beer by bacteria able to produce 4-VG is highly undesirable in most beers, although it is a normal aspect of the Bavarian *Weizenbier*, in which yeasts producing this enzyme are used to ferment worts derived from malted wheat.

Other monomeric phenolic species present in beer include catechin and quercetin [61]. Unlike ferulic acid, catechin is firmly accepted as an antioxidant through its ability both to scavenge oxygen radicals and to inhibit the enzyme lipoxygenase, which promotes the initial breakdown of unsaturated fatty acids to staling carbonyls.

When monomeric phenols are oxidized they may polymerize, yielding polyphenolic species [13]. Some of these cross-link with the proline groups in certain beer polypeptides to form insoluble complexes which are responsible for cloud (haze) in beer [62]. Haze formation is avoided by preventing oxygen ingress into beer (see earlier) and by avoiding excess levels of haze-forming polypeptides and phenolics in beer. The former can be selectively removed by silica hydrogels [63] or by precipitation with tannic acid [64]. The latter may be removed by adsorption on polyvinylpolypyrrolidone (PVPP) [65]. Just as importantly, good brewing practice ensures that these haze-forming materials don't emerge into the beer in excess. Precautions include application of vigorous wort boiling (in which heat-sensitive protein is precipitated by polyphenols) and chilling to as low a temperature as possible short of freezing (-1 °C) postfermentation (when the cold-sensitive proteins are thrown out of solution).

Tannins are highly significant for the astringent character of drinks such as cider and tea but the levels found in beer are probably too low for them to exert a significant direct effect on flavor.

Low Molecular Weight Contributors to Beer Aroma. Many people misguidedly believe that most of the flavor of beer is derived from its taste. In fact they are detecting the flavorsome materials by the nose; there are only four true characters detected on the tongue: bitterness, sweetness, sourness, and saltiness [66].

The confusion about what is detected by tongue and what by nose arises because there is a continuum between the back of the throat and the nasal passages. A beer's smell is the net effect of a complex contribution of many individual molecules. No beer is so simple as to have its aroma determined by one or even a very few substances. The perceived "nose" is a balance between positive and negative flavor notes, each of which may be due to more than a single compound from different chemical classes. Some of these volatile substances come from the malt and hops. A great many, though, are side products of the metabolism of yeast; the pathways involved in producing several classes of flavor substance pivot around pyruvate (Figure 7).

First, we need to define the term "flavor threshold" as the lowest concentration of a substance which is detectable in beer [67]. This is simplistic because low levels of individual compounds in a given class present at concentrations below their own flavor thresholds may cumulatively interact with a receptor site in the olfactory system to generate a definite response by the taster. Sometimes "flavor units" are discussed: a flavor unit is the concentration of a substance divided by its flavor threshold. Thus for phenylacetic acid, which has a flavor reminiscent of honey and a flavor threshold of approximately 2.5 mg/L, two flavor units equates to 5 mg/L. Compounds present in beer at levels of 1–2 flavor units are generally weakly detectable, whereas at higher levels a character should be readily noted.

Esters. Several esters may make a contribution to the flavor of beer, providing a fruity character. Of particular importance are ethyl acetate and isoamyl acetate. Their levels in beer are influenced by several factors, such as the sugar concentration in the wort and the amount of oxygen provided to the yeast [68]. Esters are formed from their equivalent alcohols (see later) when the acetate group is available because it is not required as the prime building block for the synthesis of key components (lipids) of the yeast membranes. Therefore, factors that promote yeast production lower ester production, and vice-versa. Of overriding importance, though, is yeast strain; some yeasts have inherently more ability to produce esters than do others. It's just the same for other low molecular weight volatiles in beer: yeast strains are therefore selected, *inter alia*, on the basis of their ability to produce certain flavors or not, depending on the character (or lack of it) which the brewer wants in a beer.

Alcohols. Easily the most important of the alcohols in beer is ethanol, with which we started this journey. The alcohols are important as the immediate precursors of the esters, which are rather more flavor-active than their parent molecules. Accordingly it is important to be able to regulate the levels of the higher alcohols produced by yeast in order that ester levels can also to be controlled.

The higher alcohols (i.e., those larger than ethanol) are produced by yeast as byproducts of amino acid metabolism [68]. For this reason the levels of amino acids available in wort influence the levels of these substances formed: the more nitrogen available to the yeast, the less it will

need to make its own amino acids. However, the situation is complex because *Saccharomyces* is also capable of synthesizing higher alcohols from the degradation products of sugar metabolism. Production of higher alcohols is increased when either too much or too little assimilable nitrogen is available to the yeast. Even more important than amino acid levels, though, is the yeast strain; ale strains produce more higher alcohols than lager strains. Conditions favoring increased yeast growth (e.g., excessive availability of oxygen) promote higher alcohol formation, but this can be countered by application of a top pressure on the fermenter.

Sourness. Any sourness in beer is due to the organic acids (including acetic, lactic, and succinic acids) that are produced by yeast during fermentation [69]. They lower pH; the H+ ion causes the sour character perceived on the palate. Higher levels of these acids are produced in vigorous fermentations.

Vicinal diketones. Whereas the esters and higher alcohols in many circumstances can make positive contributions to beer flavor, the flavor of very few beers (if any) is helped by the presence of the vicinal diketones (VDKs) diacetyl and pentanedione, which afford butterscotch and honey flavors respectively [70]. These substances are side-products of the pathways by which yeast produces certain amino acids. Precursor molecules leak out of the yeast before breaking down spontaneously to form VDKs. Fortunately, yeast can mop up the VDK provided it is healthy and stays in contact with the beer. Many brewers allow a temperature rise at the end of fermentation to speed up the removal of VDKs through an acceleration of metabolic rate. Others introduce a small amount of freshly fermenting wort as an inoculum of healthy yeast (a process known as krausening). One patented process involves accelerating the decomposition of the VDK precursors by heating beer (in the strict absence of oxygen, otherwise cooking will occur) prior to flowing it through a bed of immobilized yeast, which consumes the free VDKs [71]. Alternatively, a bacterial enzyme (acetolactate decarboxylase) can be added to a fermentation; this enzyme converts acetolactate to acetoin without the intermediacy of the much more flavor-potent diacetyl [72].

Persistent high VDK levels in a brewery's production may be indicative of an infection by *Pediococcus* or *Lactobacillus* bacteria.

Sulfur Compounds. Perhaps the most complex flavor characters in beer arise from the sulfur-containing compounds. There are many of these in beer and, in the right levels and proportions, they play a major role in determining the character of different styles of product. Many notable ales have a deliberate hydrogen sulfide character, although not one which is overdone in terms of "egginess" [73]. Lagers frequently have a more complex, sulfury character. Some have a distinct dimethyl sulfide (DMS), sweet-corn character, while some have features ranging from "cabbagy" to burnt rubber [74].

It is worth lingering on DMS for a moment, for it is the best example of how a detailed investigation of the pathways by which a molecule arises in beer has led to production strategies that allow good control over the levels of a flavor-determinant in beer.

All of the DMS in a lager ultimately originates from a precursor, *S*-methylmethionine (SMM), which is produced during the germination of barley [75]. SMM is heat-sensitive and breaks down rapidly whenever the temperature gets above about 80 °C in the malting and brewing processes. Thus SMM levels are lower in the more intensely kilned ale malts and, therefore, DMS is a character more associated with lagers. SMM dissolves in wort during mashing and is further broken down during boiling and in the whirlpool. If the boil is vigorous, most of the SMM is converted to DMS and this is volatilized. Conditions are more gentle in the whirlpool; any SMM surviving the boil will be broken down to DMS, but the fact that conditions are not turbulent means that the surface area available for DMS loss is much reduced and it tends to stay in the wort . Brewers seeking to retain some DMS in their lager specify a finite level of SMM in their malt (perhaps 6–8 μg DMS equivalents per g of malt) and will manipulate the boil and whirlpool stages in order to deliver a certain level of DMS into the pitching wort. During fermentation much DMS is driven off with the carbon dioxide produced, so the level of DMS specified in the wort is somewhat higher than that specified for beer. To increase the complexity, it is also recognized that some of the DMS produced during malt kilning is oxidized to dimethyl sulfoxide (DMSO) [76]. This substance is not heat-labile, but is water-soluble. It enters into wort at quite high levels and some yeasts are quite adept at reducing it to DMS.

Life is seldom simple—and yet further complexity is introduced by the tendency of other materials in beer to suppress the intensity of DMS character. For example, phenylethanol and phenylethyl acetate tend to interfere with the perception of DMS [77]. Quite why is so far a mystery—but it is certain that there are many such antagonisms that influence the perception of many of the flavor characters in beer.

Hydrogen sulfide (H_2S) may be produced by yeast (and strains vary considerably), by the breakdown of cysteine or glutathione, or by the reduction of inorganic sources such as sulfate and sulfite [78]. More H_2S is found in beer if the yeast is in poor condition because a vigorous fermentation purges H_2S. Any other factor which hinders fermentation (e.g., a lack of zinc or vitamins) also leads to an increase of H_2S levels in beer. H_2S is also a product of the autolysis of yeast, which occurs in unhealthy yeast.

Malty Notes. Apart from being the source of the DMS character in beer, malt can make other contributions to flavor, most of which are as yet poorly chemically defined [79]. "Malty" character is due in part to isovaleraldehyde, formed by a reaction between the amino acid leucine and reductones in the malt. The toffee and caramel flavors in crystal malts and the roasted, coffee-like notes found in darker malts are due to complex components produced from amino acids and sugars cross-reacting during kilning. Another key change during kilning is the disappearance of grassy notes, caused by *cis*-3-hexen-1-ol, *trans*-2-hexenal, *trans*-2-*cis*-6-nonadienal, and 1-hexanol.

The cross-linking of sugars and amino acids during kilning (and wort boiling) leads to the formation of melanoidins through the Maillard reaction [80]. These are the molecules that impart color to beer; darker beers are produced from malts that have been kilned to more intense regimes.

Acetaldehyde, the immediate precursor of ethanol in yeast, has a flavor threshold of 5–50 mg/L and imparts a flavor of green apples to beer [81]. In well-set-up fermentations, yeast efficiently converts the acetaldehyde into ethanol. If levels of acetaldehyde are persistently high, this suggests premature

yeast separation, poor yeast quality, or an infection by the bacterium *Zymomonas*.

The short-chain fatty acids, which can give rancid notes to beer, are made by yeast as intermediates in the synthesis of membrane lipids [82]. Therefore, the control of the levels of these acids is achieved just as for the esters (see above); under conditions when yeast needs less lipid (i.e., when it needs to grow less), short chain fatty acids accumulate.

Concluding Remarks

It is some 8,000 years since beer was first brewed in ancient Babylon. The drink then would have born little resemblance to the product of today, which is brewed to achieve true consistency by the application of controlled technology founded on in-depth scientific understanding of the malting of barley, extraction of malt, chemistry of hop utilization, fermentation behavior of yeast, and the myriad of molecules that interact to yield beer as we know it. Beer is a nutritious beverage that brings pleasure to millions across the world. It is indeed the drink of moderation.

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