Chapter 7

Biochemistry of Brewing

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Chapter Outline

I. INTRODUCTION

This chapter reviews the brewing of beer. The purpose of brewing is to hydrolyze starch from barley malt, together with wheat, maize, rice, sorghum, unmalted barley, and sugar/syrups into a sugary nitrogenous fermentable liquid called wort and to convert it into an alcoholic carbonated beverage using yeast. Although brewing is essentially a biochemical process it also involves a number of other disciplines. This chapter largely focuses on the enzyme reactions involved in beer brewing.

Saké (a high-alcohol rice-based beverage) is also brewed and similar to beer; however, it is not distilled and will not be considered here. Beer is the world's most widely consumed (Steiner, 2009) and probably the oldest alcoholic beverage, and is the third most popular drink overall, after water and tea (Priest and Stewart, 2006).

In 2009, 1800 million hl of beer was produced globally (1 hl ≡ 100 liters). The largest beer producer in 2009 was China (418 million hl), followed by the USA (234 million hl), Russia (110 million hl), Brazil (107 million hl), and Germany (98 million hl). Beer is produced by the brewing and fermentation of starches and sugar (sucrose), mainly derived from cereal grains, commonly malted barley together with wheat (sometimes malted), maize (corn), rice, sorghum (malted and unmalted), and unmalted barley. Most beer is flavored with hops, which add bitterness and aroma and can act as a preservative, although other flavorings such as herbs or fruit may occasionally be included. Beer brewing dates from 8000 BC and the earliest known chemical evidence dates from approximately 3500 BC from the site of Godin Tepe in the Zagros Mountains of western Iran.

The beer production process is outlined in the flow diagram in Figure 7.1. Malting, mashing, and fermentation/aging are essentially enzymatic processes. The main raw material, malt, contains extract components (starch, proteins, etc.) and enzymes (amylases, proteases, etc.). However, malt is an expensive raw material. Consequently, when microbial enzymes produced by fermentation became commercially available on a large scale in the 1960s, both brewers and enzyme producers began research into substituting malt with unmalted raw materials and exogenous enzymes. With some notable exceptions, this search was unsuccessful, although, as discussed later in this chapter, it has been reviewed

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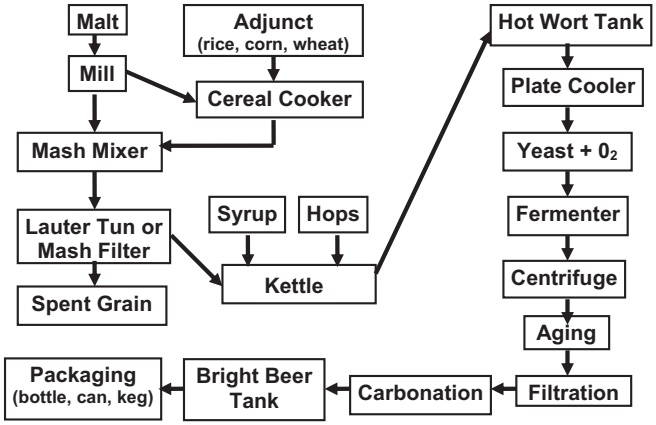


FIGURE 7.1 The brewing process.

and proposals are afoot to reintroduce 'barley brewing', as it has become known. This research has led to a range of applications for exogenous enzymes in brewing which has brought economic and technical benefits:

- improved control of the brewing process
- accelerated production resulting in increased brewing capacity
- potential for new and different beer types
- use of less expensive and unconventional raw materials.

However, there are still some unanswered questions when using exoenzymes in this context, including beer flavor match, product stability (in particular physical, flavor, and foam stability), and drinkability.

The purpose of brewing is to hydrolyze the starch/protein sources into a sugary nitrogenous fermentable liquid called wort, and to convert the wort into the alcoholic carbonated beverage known as beer in a fermentation process effected by yeast, followed by maturation. Brewing was one of the earliest biological processes to be undertaken on a commercial scale and it became one of the first processes to develop from a craft into a technology. Beer production is a unit process divided into five distinct, but related, interconnected stages:

- Malting is the germination of barley or other cereal and drying (or kilning) of the germinated cereal. Malt is made in malting plants which are usually, but not always, physically separate from breweries. The raw materials are specially selected brewing barley varieties, suitable for malting. The objective of malting from a brewing (and distilling) perspective is to permit the development of enzymes that will hydrolyze proteins and starch during the later stages of germination and subsequently during mashing.
- Mashing involves the hydrolysis of proteins/peptides, starch, and other materials from the ground malted barley and unmalted cereals (adjuncts) by a spectrum of enzymes (we will discuss this in more detail later, in Section III) to produce a water-soluble, largely fermentable extract which can be separated from the insoluble material (called spent grains). This unboiled, unhopped non-sterile liquid is called sweet wort.
- Wort boiling, with the inclusion of hops, or hop extracts, and sometimes sugar and/or syrups to produce a sterile medium called wort.
- Fermentation with yeast, followed by maturation and filtration.
- Packaging, used generally to mean kegging, bottling, and canning.

Brewing is essentially a biochemical process, but also involves many other disciplines such as microbiology and botany (both closely related to biochemistry), analytical, organic and inorganic chemistry, process engineering and control, structural engineering, separation systems including adsorbants and filtration, taste and flavor assessment, statistics, and physics (heat, refrigeration, etc.). Biochemistry is essentially a study of the biochemical processes in living organisms (in the case of brewing, plants and yeast). It deals with the structure and function of cellular components such as proteins, carbohydrates, lipids, nucleic acids, and other biomaterials. Perhaps the most important aspect of the biochemistry of brewing is that all biological reactions involve enzymes. Therefore, this chapter will focus on the enzyme reactions that are involved in the beer brewing process. There are many chemical reactions during brewing that do not involve enzymes, such as the isomerization of hops during wort boiling, the formation of coloring materials (particularly melanoidins, as discussed in Chapter 6) during green malt kilning and wort boiling,

the development of skunky (light struck) off-flavors when beer is exposed to light, and the generation of haze and staling off-flavors when beer is stored for prolonged periods. These chemical reactions will not be discussed in detail in this chapter; however, further details can be found in Bamforth (2009).

The principal raw materials used in the brewing process are malted cereals (usually barley, but not always; sometimes wheat and sorghum), unmalted cereals (corn, wheat, rice, sorghum, oats, barley), sugar and syrups (usually called adjuncts), hops, water, and yeast (Figure 7.2). In addition, there are various additives (including some enzymes) and processing aids which will be discussed in this chapter if they involve biochemical reactions. Some processing aids and additives take part in chemical reactions, for example, silica gel and polyvinylpolypyrrolidone (PVPP) (Leiper *et al.*, 2003).

II. MALT AND MALTING

The objectives of malting are to develop a spectrum of enzymes which hydrolyze the constituents of barley malt (and other cereals) to develop a fermentable extract called wort. Wort is a medium that will support yeast growth and fermentation with beer as the end product. It is important that beer is drinkable (beer is not usually sipped, it is drunk!) and it exhibits a number of stability properties, i.e. flavor, physical, foam, and biological characteristics (Stewart, 2004). Malt contributes a large number of materials to wort (Briggs, 1998a). The principal components are free amino nitrogen (FAN) and fermentable sugars.

FAN is the sum of the individual wort amino acids, ammonium ions, and small peptides (dipeptides and tripeptides). FAN is an important general measure of yeast nutrients which constitute the yeast assimilable nitrogen during brewery fermentations (Briggs, 1998b). One important aspect of barley and malt studies is the relationship between the level of nitrogen incorporated into barley during growth in the field and the level of enzyme activity developed in barley during malting (Jones and Pierce, 1965). During the malting of barley grains, large-molecular-weight components of the endosperm cell walls, the storage proteins, and the large and small starch granules in the endosperm are hydrolyzed (modified) enzymatically by, for example, amylases, proteases, and glucanases, rendering them more soluble in hot water during mashing.

The word 'malt' is derived from the Anglo-Saxon *mealt* and perhaps has the same root as melt, referring to grain softening that occurs during germination, or *malled* (mauled: broken or ground), as malts are milled before being used in brewing (and distilling) (Singer *et al.*, 1954). Malting is the limited germination of cereal grains, usually, but not always, barley. Sometimes malt is used 'green', meaning undried and not kilned. Although malt made from barley is by far the most important, it is also made from wheat, rye, oats, triticale, maize (corn), sorghum, various millets, and rice.

Malting is perhaps the oldest biotechnology. The cultivation of barley and wheat probably began around 10,000 BC (Briggs, 1998c) and wild grain must have been collected earlier. Malting is the controlled germination of cereals, followed by termination of this natural process by the application of heat to dry the grain (kilning). Further heat is then applied to kiln the grain in order to produce the required flavor and color. According to the Brewing and Malting Research Institute (BMBRI) based in Winnipeg, Canada, the following characteristics are required to produce superior malting barley:

- pure lot of an acceptable variety
- germination of 96% grains or higher

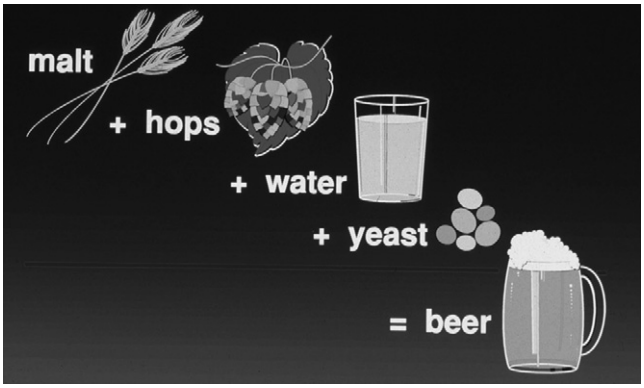


FIGURE 7.2 Principal brewing raw materials.

- no evidence of preharvest germination
- protein concentration of 11−12.5% on a dry weight basis
- maximum moisture content of 13%
- plump kernels of uniform size
- free from disease, mycotoxins such as deoxynivalenol (DON), and chemical residues
- free of frost damage and weathering
- less than 5% peeled or broken kernels
- free of insects, ergot, treated seeds, smut, and odor.

The stages of malting are usually divided into steeping, germination, and kilning. In reality, malting involves more than this and the divisions between these 'classical' stages are not clear-cut. Before the production process can begin, barley must be obtained by the maltster, either directly or indirectly from the farmer, and it must be clean and stored ready for use. Prior to this, farmers must have been persuaded to grow acceptable malting barley varieties that will meet malting quality standards. In turn, barley breeders must have developed these varieties. After the kilning process, malt must be cleaned ('dressed') and stored for a minimum period before blending and transportation to a brewer (or distiller). By-products of the process, including broken grains, malt sprouts (culms), and dust, are collected and sold for use as animal feed. Increasingly, maltsters blend culms and pelletize them. When grain is converted into malt, some malting losses are inevitably by-products.

The malting process is a blend of pure and applied science involving plant and microbial biochemistry, physiology, chemistry, physics, and engineering. The stages of a typical malting process are depicted in Figure 7.3. However, there are several variations on the basic procedure. The procedure employed is based on the principle that barley, or other grain, must be converted into malt of the best achievable quality, economically, in the shortest feasible time, with the best yield. The choice of malting procedure is guided by these considerations. However, it is beyond the scope of this chapter to discuss the detailed nuances of malting. The focus is the biochemistry of the process which includes the major metabolic pathways that operate during grain germination. These pathways include hundreds of compounds that are interconverted under the influence of a large number of enzymes and provide routes by which the carbon skeletons of carbohydrates, amino acids, and lipids can be interconverted. In addition, highly polymeric substrates, such as polysaccharides and proteins, can provide respiratory fuel and building blocks for polymeric substances, such as cell wall components. The grain tissues undergo changes as malting proceeds. The

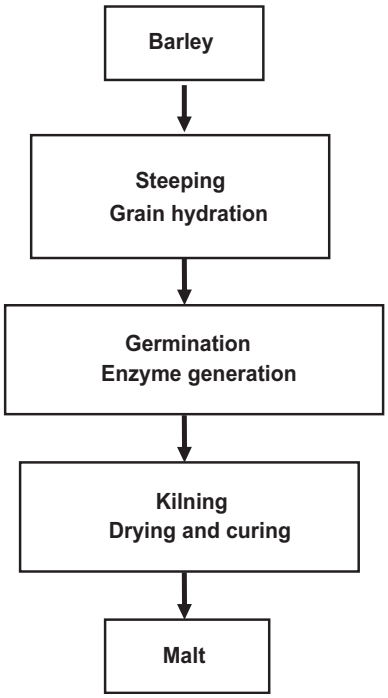


FIGURE 7.3 Typical barley malting process.

aleurone cells are partly depleted of their contents but are metabolically active. The contents of the dead starchy endosperms are partly degraded and depleted. The embryo metabolizes and grows, chiefly at the expense of hydrolysis products from the starchy endosperm. The hydrolysis and biosynthetic processes proceed simultaneously. There is a net breakdown of polymeric substances, such as starch, and a migration of substances from the aleurone layer and the endosperm to the embryo.

Although the changes that occur during malting are normally described in terms of physical modification of the grain and alteration in conventional malt analysis (Table 7.1), on a biochemical basis, the gross changes are the net result of the degradation of reserve substances, the interconversion of substances in the living embryo and aleurone layer, the net flow of substances to the embryo from the aleurone layer and starchy endosperm, the synthesis of new grain substances, and their incorporation into the new, growing tissues (the acrospire and rootlets) of the embryo. Allowances must be made for the losses of dry matter that occur during the conversion of grain into malt. These malting losses result from:

- leaching of substances from the grain during steeping
- fermentative processes and the respiratory oxidation of substances to carbon dioxide and water
- removal of the rootlets.

The total losses during malting are usually in the range 6−12% of the original dry weight. Losses can be much larger when cereals such as wheat and sorghum are malted.

The chemical and biochemical changes that take place during malting are complex. They can only be understood by appreciating the range of reactions that occur during the overlapping processes of steeping, germination, and kilning, and the effects of deculming and dressing (cleaning) the malt. During steeping, the grain is permitted to imbibe water so as to increase the grain moisture from 12−14% to 42−48%. This occurs by immersing the grain in water or by spraying with water, or usually a combination of both. The steep water becomes dirty and is replaced at least once to keep the grain fresh. During steeping the grain swells and softens, and the living tissues resume their metabolism, which had ceased during grain ripening and drying prior to harvesting and during storage awaiting malting. Sometimes air is blown through the grain−water mixture (aeration), or the grain can be air-rested − the water is drained away and air is sucked downwards through the grain. When the grain has achieved the correct moisture content, the steeping water is removed. Usually this steeped grain is transferred to a germination vessel. In some malting plants, steeping and germination, and occasionally kilning, take place in one container.

Each batch of grain being malted is referred to as a 'piece'. Following steeping, germination begins and the grain undergoes modification. Modification is an imprecise term that signifies all the desirable changes, both biochemical

TABLE 7.1 Typical Barley Malt Analysis

Moisture (%)	3.8−4.2
Extract (%)	79.9−81.0
Wort color (°SRM)	1.4−1.7
Diastatic power (°ASBC)	120−145
α-Amylase (DM)	39−49
Malt protein (%)	10.8−12.3
Wort protein (%)	4.9−5.6
FAN (mg/l)	180−220
Wort viscosity (cP)	1.38−1.48
Wort β-glucan (mg/l)	25−150
Friabilimeter value (%)	70−86
Wort fermentability (%)	78−82

FAN: free amino nitrogen.
From Briggs (1998b).

and chemical, that occur when grain is converted to malt. Modification continues during the initial stages of kilning. The three major aspects of modification are:

- accumulation of hydrolytic enzymes (details discussed later, in Section III)
- a variety of chemical reactions occurring in the grains
- physical changes in the grain, which appear as weakening and softening.

Visible signs of germination include the initial appearance of a white chit at the end of the grain, followed by a tuft of rootlets or culms. At the same time, the acrospire (coleoptile or shoot) grows. It is covered by the husk in the barley but it grows freely in many other grains. The germinated grain is transferred to the kiln while it is still fresh (called green or undried, but not green colored). The moisture content of kilned barley malt is usually 4−4.5%, reduced from 42−48%.

Biochemical changes occur in malting grains' progress as modification in the starchy endosperm occurs. A partly malted grain retains some of its barley character. In each batch of grain there is heterogeneity in the sense that individual grains vary in size, shape, maturity, chemical composition, and potential for generating enzymes. Furthermore, individual grains modify at different rates and receive different treatments during steeping, germination, and kilning.

A. Grain Structure

Barley grains (Figure 7.4), except for naked (huskless) barley, contain husk, pericarps, testa, aleurone layer, starchy endosperm, and embryo (Briggs, 1998b). In terms of total dry weight of the barley grain, the husk is 10−12%, the pericarp and testa 2−3%, the aleurone layer 4−5%, the starchy endosperm 77−80%, and the embryo 2−3%.

The husk is composed of two leaf-like structures. The dorsal half is called the lemma and the ventral half is the palea. The husk protects the underlying structure of the grain, especially the embryo. Husk damage is regarded as unacceptable and barley samples are rejected if husk damage is beyond specification requirements. High damage implies embryo damage, uncontrollable embryo growth, and mash filter operational difficulties. The husk contains background levels of microorganisms such as fungi and bacteria. These microorganisms may have invaded the grains in the field before harvesting or during storage before malting (Flannigan, 1996). The pericarp is the fruit of the grain. Cereal grains are fruits and, strictly speaking, should not be referred to as seeds. Similar to the husk, the pericarp contains a waxy cuticle, and below this waxy layer is a compressed structure of cells. The pericarp is semipermeable, so certain chemicals will pass through it, while others, such as the plant hormone gibberellic acid, will not. Water can pass through the pericarp. Damage to the pericarp during abrasion (a process whereby 7−9% of a grain's weight is separated as hulls, mainly husk, with minimal reduction in germination or grain breakage) allows gibberellic acid to enter the aleurone layer directly, rather than via the germinated embryo, thereby improving modification of the starchy endosperm by improving the efficiency of the aleurone in producing endosperm-degrading enzymes (Sandegren and Beling, 1959). However, abrasion is not currently used to any great extent.

The testa comprises two lipid layers that enclose cellular material. The testa is permeable to gibberellic acid. Phenolic compounds such as anthocyanogens (proanthocyanogens) are associated with the aleurone and testa and can be clearly seen in some varieties of barley and sorghum. The small area of the pericarp−testa that lies over the coleorhiza (chit) is called the micropyle. The latter may facilitate the uptake of water and salts into the embryo during germination.

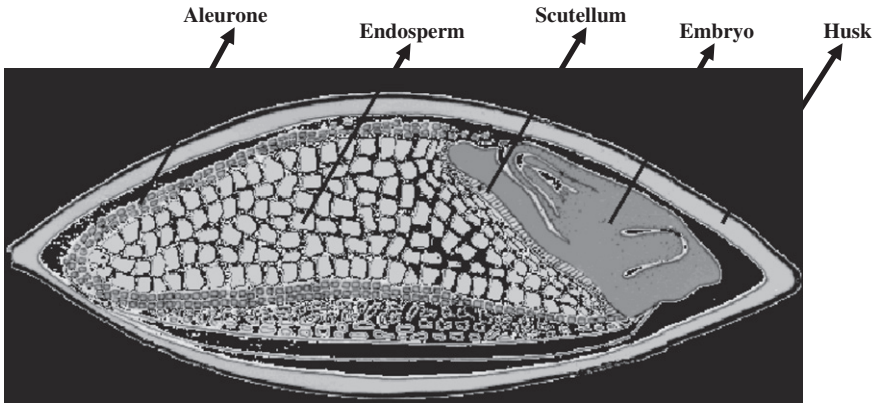


FIGURE 7.4 The barley grain. *(From Briggs, 1998c.)*

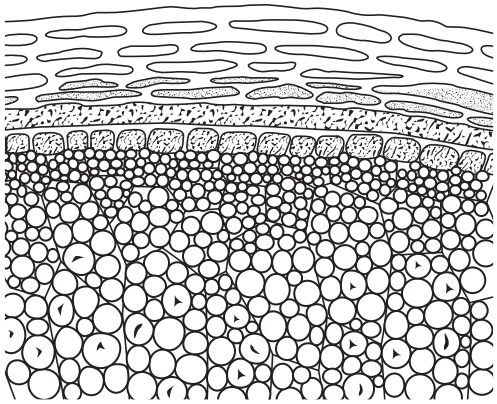


FIGURE 7.5 Structure of the barley aleurone layer. *(From Briggs, 1998c.)*

The aleurone layer is two or three cells deep over the starchy endosperm (Figure 7.5). It extends over the embryo as a single cell layer. Therefore, excised embryos contain alerone cells and produce endosperm-degrading enzymes. During malting, gibberellic acid from germinated embryos can induce alerone cells to produce endosperm-degrading enzymes such as α-amylase, endo-β-1,3:1,4-glucanases, limit dextrinases, endoproteases, and xylanases (pentosanases). It has been established that α-amylase, endoprotease, and limit dextrinase are produced *de novo* and aleurone layers stimulated by gibberellic acid.

III. MASHING AND BOILING

The purposes of mashing are:

- to extract starch, proteins, peptides and other components from the malt
- to render the extract fermentable by ensuring the necessary enzymatic hydrolysis of the above components to sugars, amino acid, small peptides, etc.

Before mashing, the malt is milled using a variety of milling procedures. This is a physical process and therefore beyond the scope of this chapter (Briggs, 1998a). The milled grain is wetted to stimulate enzyme activity which will hydrolyze the starch into fermentable sugars, the proteins into amino acids and small peptides, and the lipids into free fatty acids and sterols. In order to achieve this complex hydrolysis procedure, the mash is subjected to a series of heating and rest periods at set temperatures to realize the optimum catalytic conditions with reference to the type of beer being produced. The infusion mashing program is extensively used and is shown in Figure 7.6. The initial temperature of 52°C is used to stimulate both protease and glucanase activity (often called the protein rest) but the rates of activity of the two enzyme systems are unclear. After 15−20 minutes the temperature is increased gradually

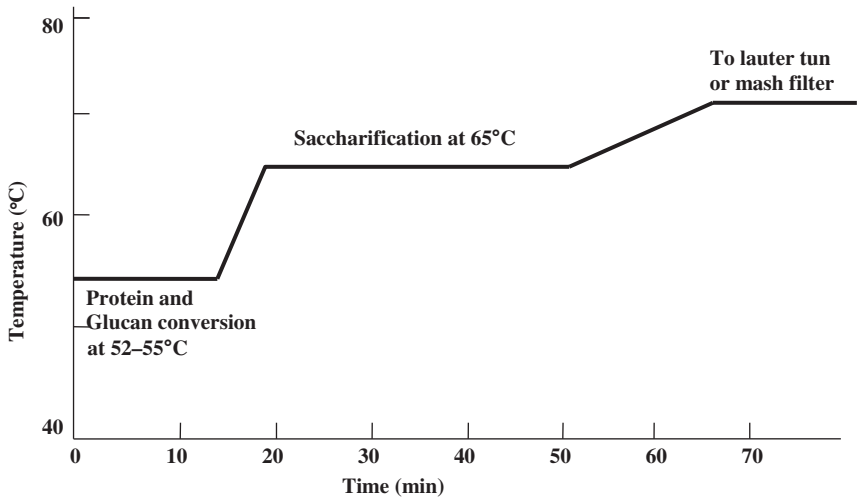


FIGURE 7.6 Temperature profile of a typical infusion mashing program.

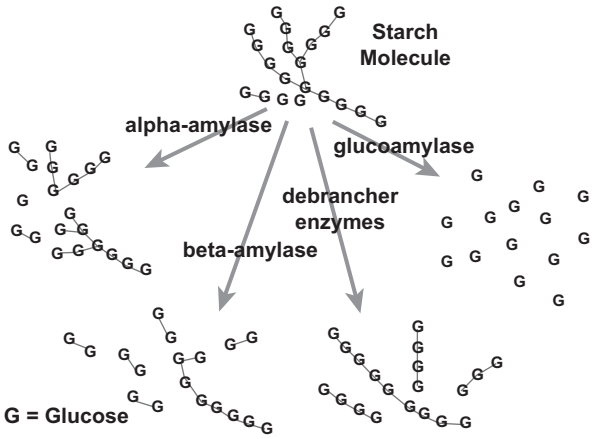
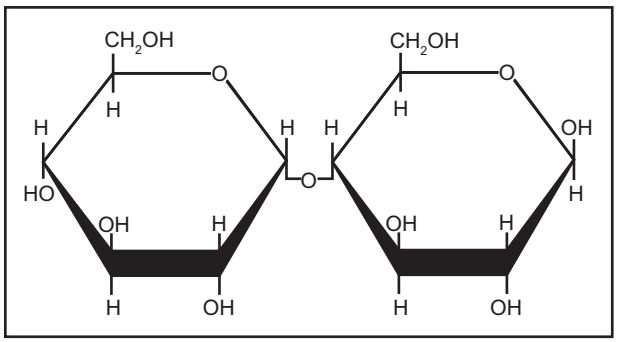


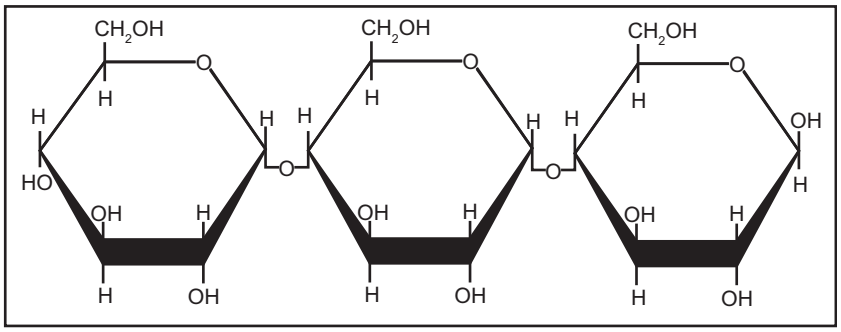
FIGURE 7.7 The enzymatic hydrolysis of starch.

to 65°C. This temperature is the saccharification temperature and is optimal for both α- and β-amylase activity. α-Amylase is an endoamylase that hydrolyzes 1,4-α-glucosidic linkages in amylose (contains 1→4 linkages) and amylopectin (contains both 1→4 and 1→6 linkages). This enzyme is virtually absent from mature barley unless it has pregerminated. However, considerable quantities are synthesized *de novo* in the embryo and aleurone layer and large proportions are generated into the starchy endosperm. β-Amylase is an exoenzyme that catalyzes the hydrolysis of the 1→4 linkages penultimate to the non-reducing chain ends (Figure 7.7), releasing the disaccharide maltose (Figure 7.8) and an oligosaccharide (also called dextrin) shortened by the removal of two glucose residues. Unlike α-amylase, this enzyme is present in unmalted barley. During malting, the level of free β-amylase may initially fall

FIGURE 7.8 Structure of maltose and maltotriose.



Maltose,
Molecular weight = 342



Maltotriose,
Molecular weight = 504

during steeping and subsequently, during germination, nearly all the β-amylase becomes free, and the bound form disappears. This enzyme will not attack 1→6 or 1→4 linkages immediately adjacent to each other.

Another important enzyme action during mashing is the debranching enzyme that hydrolyzes 1→6 linkages in amylopectin, dextrins, and other oligosaccharides. This enzyme is also known as limit dextrinase and as pullulanase because of its ability to degrade a particular bacterial polysaccharide, namely pullulan. In malt, this enzyme occurs in both freely soluble and bound forms. There exists a heat-stable protein in barley that inhibits malt limit dextrinase (Briggs, 1998c). The amount of inhibition increases during malting. However, there is evidence that this enzyme is synthesized *de novo* in the barley aleurone layer during germination.

After approximately 30 minutes at 65°C (saccharification rest) the temperature is raised to 78°C: the mash-off temperature. The principal purpose of this mash-off temperature is to inactivate most of the enzymes that were active in the mash. In addition, some final α-amylolysis will occur at this temperature, whereas β-amylase will be rapidly inactivated. Also, at this temperature, the viscosity of the sweet wort (due to β-glucans and arabinxylans) will be reduced and many, although not all, of the microorganisms contaminating the malt will be heat inactivated. This sweet wort is separated from the spent grains in a lauter tun or mash filter.

The sweet wort is boiled in a kettle (also called a copper), usually for 30−60 minutes with 4−6% evaporation. Boiling is needed to isomerize the hop α-acids (isomerized hop acids are bitter whereas non-isomerized hop α-acids are not), to strip out unwanted malt and hop volatiles, to denature proteins and coagulate proteins/polyphenols as hot break, and to fix the wort composition (see Section IV) by terminating all enzymatic and microbiological activity surviving the mashing process. As a consequence of boiling and evaporation, there will also be color development and an increase in wort gravity that need to be accommodated to achieve the finished target for a particular wort. Ideally, a kettle should be fitted with in-line probes to follow the isomerization of α-acids, the disappearance of dimethyl sulfide, the coagulation of colloidal size particles (0.1−10 μm) to form hot break (30−70 μm), and the wort gravity. Currently, this in-line control is not practical and the brewer has to rely on pragmatic experience to achieve these critical parameters.

IV. WORT COMPOSITION

Compared to other media used in the production of fermentation alcohol (both industrial and potable), wort is by far the most complicated. Therefore, when yeast is pitched (inoculated) into wort it is introduced into a complex environment because it consists of simple sugars, dextrins, amino acids, peptides, proteins, vitamins, ions, nucleic acids, and other constituents too numerous to mention. One of the major advances in brewing science during the past 40 years has been the elucidation of the mechanisms by which the yeast cell utilizes, in an orderly manner, the plethora of wort nutrients. Wort sugars (Stewart, 2006) and amino acids (Jones and Pierce, 1964), as discussed in Subsection D, Wort Free Amino Nitrogen, below, are removed in a distinct order at various points in the fermentation cycle.

A. Wort Sugars

Wort contains the sugars sucrose, fructose, glucose, maltose, and maltotriose, together with dextrin material. A typical percentage sugar spectrum of brewer's wort is shown in Table 7.2. In the normal situation, brewing yeasts are

TABLE 7.2 Typical Sugar Spectrum of Wort

Sugar	Composition (%)
Glucose	10−15
Fructose	1−2
Sucrose	1−2
Maltose	50−60
Maltotriose	15−20
Dextrins	20−30

From Stewart (2006).

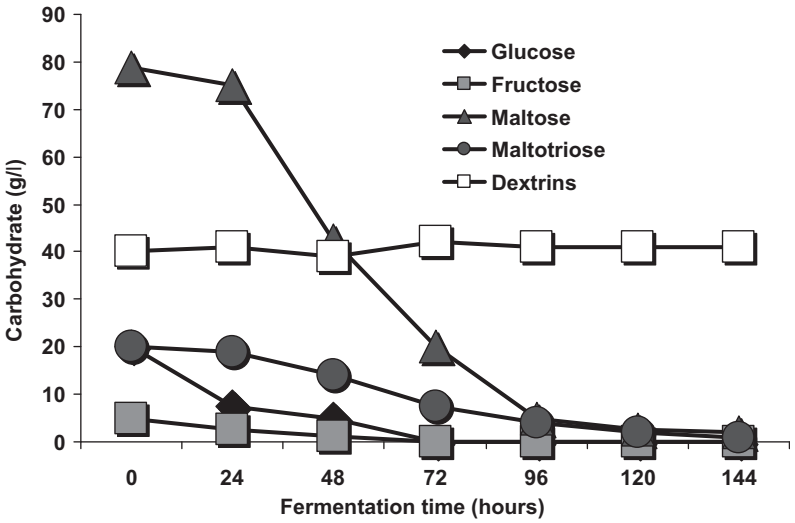


FIGURE 7.9 Order of uptake of wort sugars by the yeast cell. *(From Stewart, 2006.)*

capable of utilizing sucrose, glucose, fructose, maltose, and maltotriose in this approximate sequence (or priority), although some degree of overlap does occur, leaving maltotetraose (G4) and the larger dextrins unfermented (Figure 7.9).

The objectives of wort fermentation are to consistently metabolize wort constituents into ethanol, carbon dioxide, and other fermentation products in order to produce beer with satisfactory quality and stability. Another objective is to produce yeast crops that can be confidently repitched into subsequent brews (Stewart and Russell, 1986).

Saccharomyces cerevisiae strains, including brewery yeast strains, are single-celled fungi (more details in Subsection IV, C, below). The requirements of an acceptable brewer's yeast strain are: 'In order to achieve a beer of high quality, it is axiomatic that not only the yeast be effective in removing the required nutrients from the growth/fermentation medium (wort), able to tolerate the prevailing environmental conditions (for example, ethanol tolerance), and impart the desired flavor to the beer, but the microorganisms themselves must be effectively removed from the wort by flocculation, centrifugation and/or filtration after they have fulfilled their metabolic role' (Stewart and Russell, 1986).

These strains have the ability to take up and ferment a wide range of sugars, including sucrose, glucose, fructose, galactose, mannose, maltose, and maltotriose. In addition, *Saccharomyces diastaticus* (a subspecies of *S. cerevisiae*) is able to utilize dextrin material due to the secretion of glucoamylase. The initial step in the utilization of any sugar is usually either its passage intact across the cell membrane, or its hydrolysis outside the cell membrane, followed by entry into the cell by some or all of the hydrolysis products (Figure 7.10). Maltose and maltotriose are examples of sugars that pass intact across the cell membrane, whereas sucrose and dextrins are hydrolyzed by extracellular enzymes [invertase for sucrose and glucoamylase (amyloglucosidase) for dextrins] and the hydrolysis products are

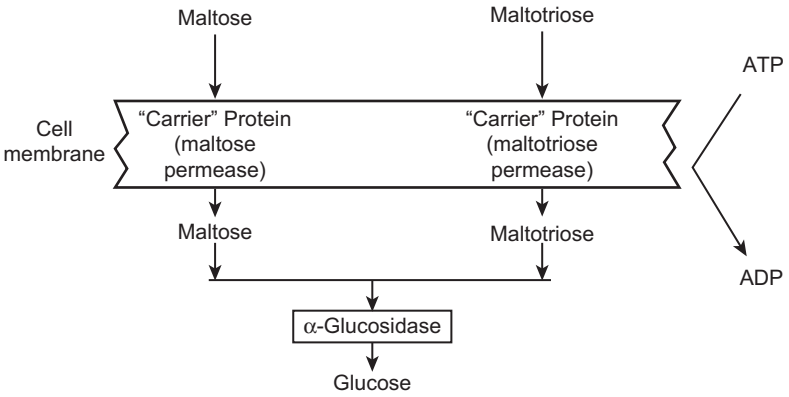


FIGURE 7.10 Uptake and metabolism of maltose and maltotriose by the yeast cell. *(From Stewart, 2006.)*

taken up into the cell. An important metabolic difference between the uptake of monosaccharides such as glucose and fructose disaccharides such as maltose or maltotriose uptake is that energy (ATP conversion to ADP) is required for maltose and maltotriose uptake (active transport) whereas glucose and fructose are taken up passively with no energy required (Bisson *et al.*, 1993). As maltose and maltotriose (Figure 7.8) are the major sugars in brewer's wort, the ability of a brewing yeast to use these two sugars is vital and depends upon the correct genetic complement. Brewer's yeast possesses independent uptake mechanisms (maltose and maltotriose permeases) to transport the two sugars across the cell membrane into the cell. Once inside the cell, both sugars are hydrolyzed to glucose units by the α-glucosidase system (Figure 7.10). The transport, hydrolysis, and fermentation of maltose are particularly important in brewing, distilling, and baking since maltose is the major sugar component of brewing wort, spirit mash, and wheat dough. Maltose fermentation in brewing, distilling, and baking yeasts requires at least one of five unlinked *MAL* loci, each consisting of three genes encoding the structural gene for α-glucosidase (maltase) (*MALS*) maltose permease (*MALT*) and an activator whose product coordinately regulates the expression of the α-glucosidase and permease genes. The expression of *MALS* and *MALT* is regulated by maltose induction and repression by glucose. When glucose concentrations are high (> 10 g/l) the *MAL* genes are repressed and only when 40−50% of the glucose has been taken up from the wort will the uptake of maltose and maltotriose commence (Figure 7.9).

B. Effect of Osmotic Pressure and Ethanol on the Uptake of Wort Glucose, Maltose, and Maltotriose

High-gravity worts (> 16°Plato, where 1°Plato ≡ 1 g sucrose dissolved in 100 ml distilled water at 20°C) have been shown to exact a negative effect upon fermentation performance (Casey *et al.*, 1984). High-gravity brewing is a procedure that employs wort at higher than normal concentration and thus requires dilution with water (usually specially treated, including being deoxygenated) at a later stage in processing. By this means, increased production demands can be met without expanding brewing, fermenting, and storage facilities. This process also enhances the sustainability of brewing because of a reduction in the amount of water and energy required in a brewhouse per unit of sales-gravity beer produced. In addition, high-gravity conditions can result in a reduction in labor, cleaning, and effluent costs (Stewart, 1999).

Although the high-gravity process has a number of advantages, inevitably there are disadvantages. Owing to the more concentrated mash (increased ratio of carbohydrate to water in order to produce high-gravity wort), there is a decrease in brewhouse material efficiency. This problem can be overcome by the use of modern mash filters (Andrews *et al.*, 2011) instead of traditional lauter tuns and/or kettle syrups, where entry of carbohydrate into the process bypasses the process whereby the unboiled wort is separated from the spent grains. In addition, the more concentrated wort during boiling results in reduced isomerization of hops, leading to decreased hop utilization. This process disadvantage can be alleviated by the use of kettle and postfermentation hop extracts.

Another major disadvantage to the high-gravity brewing process is reduced foam stability (head retention) in the final diluted beer. This aspect will not be discussed in detail here, suffice to say that the major reason is greater loss of hydrophobic polypeptides during the brewing process. This is due to reduced extraction of these polypeptides during mashing and their hydrolysis by yeast proteases during fermentation (Cooper *et al.*, 1998).

Another negative effect of high-gravity worts is their effect on overall yeast activity and performance. One of the negative influences is elevated osmotic pressure (Pratt *et al.*, 2003). This was simulated by employing the non-metabolized sugar sorbitol in a synthetic medium. Fermentations were conducted with peptone−yeast extract containing 25% (w/v) sorbitol to which was added 2.5% (w/v) glucose, maltose, or maltotriose. The effect of the elevated osmotic pressure on the transport of these three sugars is shown in Figure 7.11 as a percentage of the untreated control that did not contain added sorbitol, with both an ale and a lager yeast strain. The sugar uptake rates of the three sugars were inhibited in both strains in the presence of sorbitol, but to a much greater extent with maltose and maltotriose than with glucose. Similar effects were obtained on the uptake of glucose, maltose, and maltotriose following treatment with 10% (v/v) ethanol (Stewart, 2006) (Figure 7.12). These effects are probably a reflection of the different transport mechanisms for glucose compared to maltose and maltotriose.

Yet another disadvantage of high-gravity brewing is that the flavor of the diluted beer is often not comparable to sales-gravity brewed beer. This is primarily due to disproportionate levels of beer acetate compounds such as ethyl acetate and isoamyl acetate (Table 7.3). Varying the wort sugar source (Younis and Stewart, 1999) has been reported to modify the levels of many metabolites, including esters, although reasons for these differences are unclear. Initially, 4% (v/v) glucose and maltose in a synthetic medium (yeast extract−peptone) were fermented separately with shaking at 21°C and the production of ethyl acetate and isoamyl acetate was monitored (Table 7.4). The

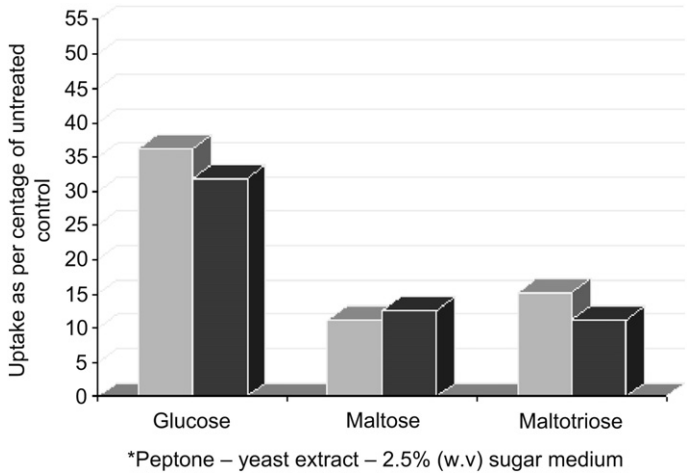


FIGURE 7.11 Effect of osmotic pressure [25% (w/v) sortibol*] on the uptake of glucose, maltose, and maltotriose by a lager strain (gray bars) and an ale strain (black bars). *(From Stewart, 2006.)*

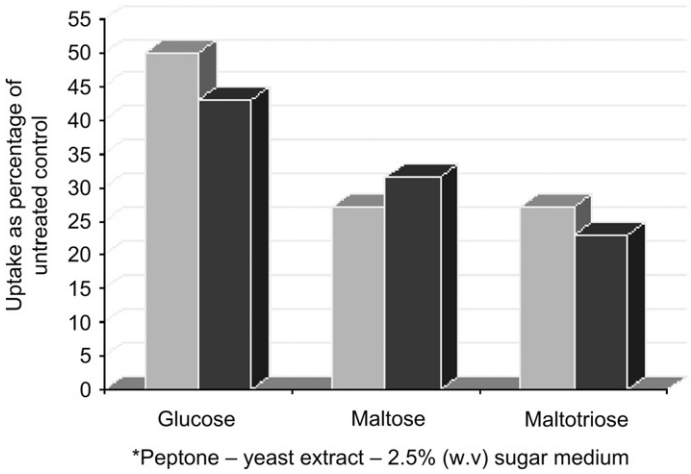


FIGURE 7.12 Effect of ethanol [10% (v/v) ethanol*] on the uptake of glucose, maltose and maltotriose by a lager strain (gray bars) and an ale strain (black bars). *(From Stewart, 2006.)*

fermentation performances of the three ale and three lager brewing strains employed were similar. In all six strains studied, the strains cultured in maltose consistently produced lower levels of both esters when in the glucose medium.

The lower levels of the esters produced with maltose as the substrate compared to glucose could be due to a number of reasons. It is possible that fermentation with maltose inhibits the transport of esters out of the cell, perhaps by modifying the plasma membrane, thus giving the impression that fewer esters are produced. Another possibility is that maltose metabolism produces lower levels of acetyl-coenzyme A which, it has been suggested, results in fewer esters due to a lack of substrate. It has been proposed that ester production is linked to lipid

TABLE 7.3 Influence of Wort Gravity on Beer Ester Levels

	12°Plato	20°Plato
Ethanol (v/v)	5.1	5.0
Ethyl acetate (mg/l)	14.2	21.2
Isoamyl acetate (mg/l)	0.5	0.7

From Younis and Stewart (1999).

TABLE 7.4 Ethyl Acetate and Isoamyl Acetate Produced by Brewing Yeast Strains During Fermentation of Synthetic Media[a]

	Ethyl Acetate (mg/l)		Isoamyl Acetate (mg/l)	
Strain	Glucose	Maltose	Glucose	Maltose
Ale 1	4.13	2.79	0.14	0.14
Ale 2	2.97	2.59	0.06	0.04
Ale 3	3.13	2.71	0.05	0.03
Lager 1	6.00	5.22	0.22	0.21
Lager 2	3.75	3.28	0.26	0.22
Lager 3	4.13	3.51	0.23	0.17

[a]*Peptone–yeast extract–4% sugar medium.*
From Younis and Stewart (1999).

metabolism (Stewart, 2005). If this is the case, or if for some reason maltose metabolism produces fewer toxic fatty acids, it would be reasonable to assume that fewer fatty acids would also be produced.

It is generally agreed that a reduction in beer ester levels, particularly ethyl acetate and isoamyl acetate, from high-gravity brewed beers would be welcome. In order to study the influence of maltose and glucose levels in high-gravity worts, two 20°Plato worts were prepared, one containing 30% maltose syrup (MS) and the other containing 30% very high maltose syrup (VHMS). The sugar composition of the two brewing syrups is shown in Table 7.5. In addition, a 12°Plato wort containing 30% maltose syrup (MS) was prepared and used as a control. The sugar spectra of the three worts are shown in Figure 7.13. The maltose plus maltotriose concentration in the 20°Plato VHMS wort had increased compared to the 20°Plato MS wort, with a corresponding decrease in the concentration of glucose plus fructose.

The three worts were fermented in the 2 hl pilot brewery of the International Centre for Brewing and Distilling at Heriot-Watt University, Scotland, UK (Figure 7.14). A lager strain was used at 13°C and the concentration of ethyl acetate and isoamyl acetate determined throughout the fermentation (Figures 7.15 and 7.16). The profiles were similar with both esters. The concentration of both esters in the 20°Plato MS fermented wort was twice that in the 12°Plato MS fermented wort, as would be expected. However, the ester concentration in the 20°Plato VHMS wort was reduced by approximately 25% compared to the 20°Plato MS wort (Younis and Stewart, 1999). These results confirm the findings using synthetic media with simple sugars that maltose fermentations produce less ethyl acetate and isoamyl acetate than glucose fermentations (Younis and Stewart, 1998).

C. Uptake of Wort Maltose and Maltotriose: Differences between Ale and Lager Yeast Strains

The commercial worldwide production of ale has always been much lower than that of lager and over the years this difference has grown. The trends in ale compared with lager consumption in Ontario, Canada, and the UK are shown

TABLE 7.5 Sugar Composition of Brewing Syrups

	MS	VHMS
Glucose	15[a]	5
Maltose	55	70
Maltotriose	10	10
Dextrins	20	15

MS: maltose (55) syrup; VHMS: very high maltose (70) syrup.
[a]*Percent composition.*
From Priest and Stewart (2006).

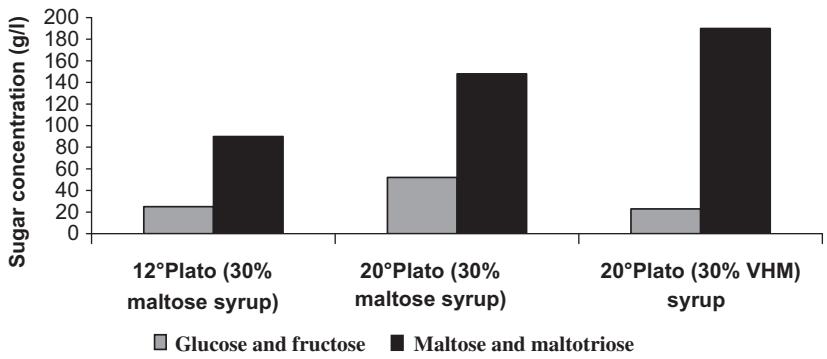


FIGURE 7.13 Wort sugar profile. *(From Younis and Stewart, 1998.)*

FIGURE 7.14 Brewing pilot plant at Heriot-Watt University, Edinburgh, UK. *(Source: author's own photograph.)*

in Tables 7.6 and 7.7, respectively. In the USA, currently 4.4% of beer produced is ale, largely due to the craft brewing sector.

There are several differences in the production of these two types of beer, one of the main ones being the characteristics of ale and lager yeast strains. Considerable research by many breweries and institutions on this topic has been conducted (Barnett, 1992; Pederson, 1995; Pulvirenti *et al.*, 2000; Rainieri *et al.*, 2003) and the typical differences between ale and lager yeast strains have been established (Table 7.8).

With the advent of molecular biology-based methodologies, gene sequencing of ale and lager brewing strains has shown that they are interspecies hybrids with homologous relationships to one another and also to *Saccharomyces*

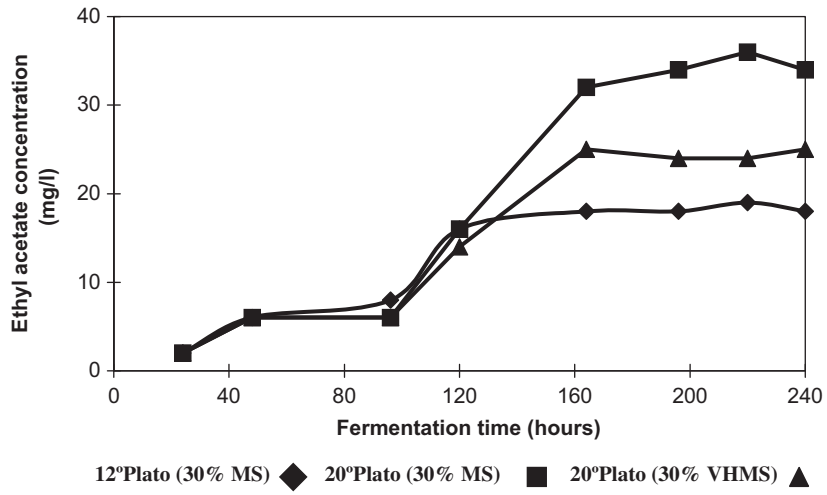


FIGURE 7.15 Ethyl acetate concentration in fermenting worts of differing gravities and sugar composition. (*u*) 12°Plato (30% maltose syrup); (*v*) 20°Plato (30% maltose syrup); (σ) 20°Plato (30% very high maltose syrup). *(From Younis and Stewart, 1998.)*

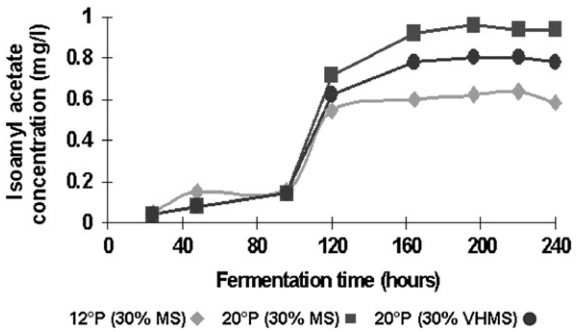


FIGURE 7.16 **Isoamylacetate concentration in fermenting worts of differing gravities and sugar composition.** *(From Younis and Stewart, 1998.)*

TABLE 7.6 Comparison of Ale versus Lager Consumption (%) Trends in Ontario, Canada (1970–2007)

Year	Ale	Lager
1970	60	40
1980	20	80
1990	10	90
2000	10	90
2005	15	85
2007	15	85

From Steiner (2009).

TABLE 7.7 Comparison of Ale versus Lager Consumption (%) Trends in the UK (1970–2007)

Year	Ale	Lager
1970	90	10
1980	70	30
1990	50	50
2000	40	60
2007	35	65

From Steiner (2009).

bayanus, a yeast species used in wine fermentation and identified as a wild yeast in brewing fermentation (Figure 7.17). The gene homology between *Saccharomyces pastorianus* and *S. bayanus* strains is high at 72%, whereas the homology between *S. pastorianus* and *S. cerevisiae* is much lower at 50% (Pederson, 1995).

Recently, a research group from Argentina, Portugal, and the USA published a paper entitled 'Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast' (Libkinda *et al.*, 2011). This confirmed that *S. pastorianus* is a domesticated yeast species created by the fusion of *S. cerevisiae* with a previously unknown species that has now been designated *Saccharomyces eubayanus* because of its close relationship to *S. bayanus*. They also report that *S. eubayanus* exists in the forests of Patagonia and was not found in Europe until the advent of trans-Atlantic trade between Argentina and Europe. This paper (Libkinda *et al.*, 2011) contains a draft genome sequence of *S. eubayanus*; it is 99.5% identical to the non-*S. cerevisiae* portion of the *S. pastorianus* genome sequence and

TABLE 7.8 Differences Between Ale and Lager Yeast Strains

Ale Yeast	Lager Yeast
Saccharomyces cerevisiae (ale type)	*Saccharomyces uvarium* (*carlsbergensis*)
	Saccharomyces cerevisiae (lager type)
	Saccharomyces pastorianus: current taxonomic name
Fermentation temperature of 18–22°C	Fermentation temperature of 8–15°C
Cells can grow at 37°C or higher	Cells cannot grow at 37°C or higher
Cannot ferment the disaccharide melibiose	Ferments the disaccharide melibiose
'Top' fermenter	'Bottom' fermenter

suggests specific changes in wort sugar and sulfate metabolism compared to ale strains that are critical for determining lager beer characteristics.

Several ale and lager yeast strains have been used to explore the mechanisms of maltose and maltotriose uptake in wort. A 16°Plato all-malt wort was used in a 30 liter static fermenter at 15°C (Figure 7.18). Under these conditions, lager strains utilized maltotriose more efficiently than ale strains, whereas maltose utilization efficiency was not dependent on the type of brewing strain (Zheng *et al.*, 1994). This supports the proposal that maltotriose and maltose possess independent, but closely linked, uptake (permease) systems (Russell and Stewart, 1980a). In addition, this consistent difference between ale and lager strains supports the observation that ale strains appear to have greater difficulty than lager strains in completely fermenting wort, particularly in high-gravity wort (Stewart *et al.*, 1995).

In order to investigate the MAL gene cassettes further, a strain with two *MAL2* and two *MAL4* genes copies was constructed using hybridization techniques. The wort fermentation rate was compared to a strain containing only one copy of *MAL2*. As expected, the overall fermentation rate with the strain containing multiple *MAL* genes was considerably faster than the strain containing the single copy *MAL2* (Figure 7.19). The principal reason for this faster fermentation rate was due to an increased rate of maltose uptake and subsequent metabolism compared to the yeast strain containing the single *MAL2* copy (Figure 7.20).

As already discussed, wort contains unfermentable dextrins (Table 7.2). These dextrins remain in the finished beer and thus give it mouth-feel and contribute to its calorific value (Brenner, 1980). To produce a low-calorie beer the dextrins must be reduced, by any of a number of techniques. One method would be to employ a yeast strain that possesses an ability to metabolize wort dextrins. The fact that there is a grouping of yeast – *S. cerevisiae* var. *diastaticus* – that is taxonomically closely related to brewer's yeast strains has already been discussed in this chapter. These strains have the genetic ability to produce an extracellular glucoamylase that can hydrolyze the dextrins to glucose which will be taken up by the yeast during wort fermentation. These genes have been identified as

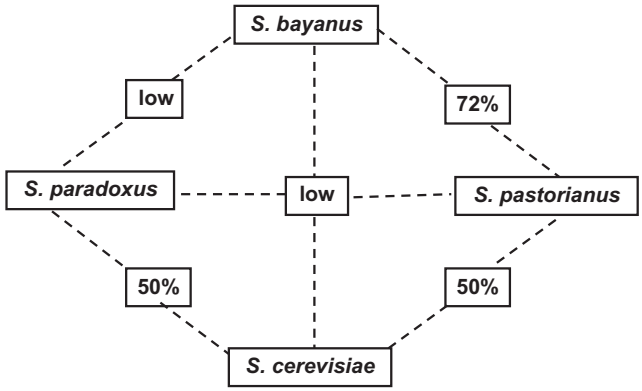


FIGURE 7.17 The *Saccharomyces sensu stricto* group for ale and lager strains. *(Adapted from Pederson 1995.)*

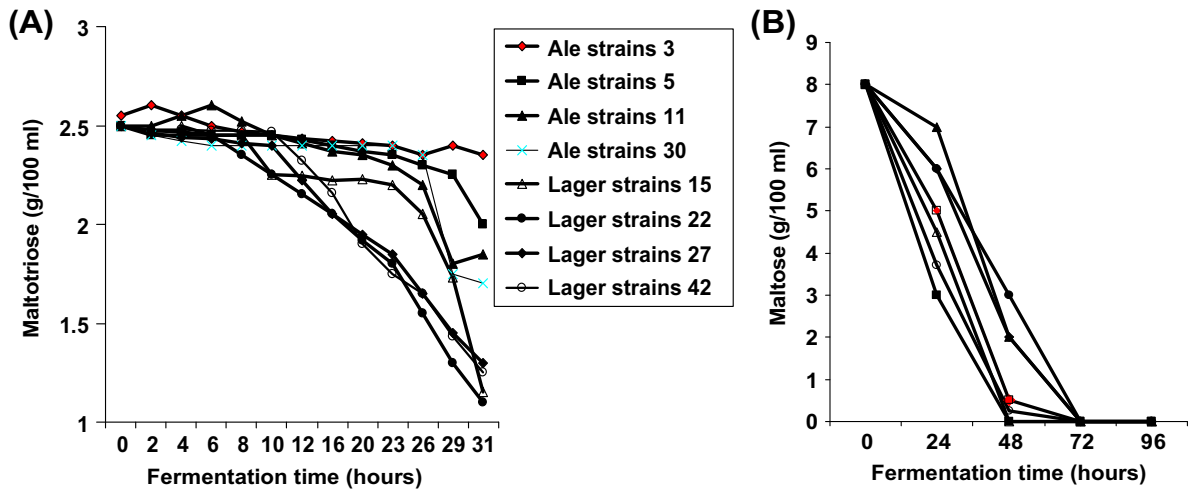


FIGURE 7.18 (A) Maltotriose and (B) maltose uptake profiles from a 16°Plato wort. *(From Zheng* et al.*, 1994.)*

a polymeric set of three genes: *STA1/DEX1*, *STA2/DEX2*, and *STA3/DEX3* (Erratt and Stewart, 1978). A yeast strain incorporating these genes was constructed and its fermentation characteristics were assessed during a wort fermentation. The amylolytic yeast exhibited a faster fermentation rate and a lower final wort degree Plato than the control yeast that was unable to metabolize wort dextrins (Figure 7.21) (Erratt and Stewart, 1981).

The extracellular glucoamylase produced by this group of yeast is thermotolerant, probably because it is heavily glycosylated (it is a mannoprotein). Therefore, the glucoamylase was not inactivated during pasteurization (12 PU ≡ 12 minutes at 60°C) of the low-dextrin beer. This resultant beer produced with an amylolytic yeast contained increasing concentrations of beer and became sweeter and sweeter (Figure 7.22). Nevertheless, a low-dextrin beer, Nutfield Lyte, produced with a glucoamylase-containing yeast, has been produced on a semi-production scale as part of a collaborative project between the Brewing Research Foundation (now Campden BRI, Brewing Division) and Heriot-Watt University (Figure 7.23). This strain has been approved for use on a production basis by the UK's Novel Food Products and Processes (Baxter, 1995). However, it is not currently being used on a production basis for brewing. Indeed, brewers are still reluctant to use genetically manipulated yeast strains (Sofie *et al.*, 2010).

A second method of producing low-dextrin beer is to use bacterial amylases (exoenzymes) that are added either to the mash mixer or to the fermenter. Further details on the use of these exoenzymes will be discussed in Section V.

Several factors in the metabolism of wort sugars during brewing and distilling have to be considered. The effects of high-gravity brewing are particularly important and some aspects of this area will be reviewed in the next subsection.

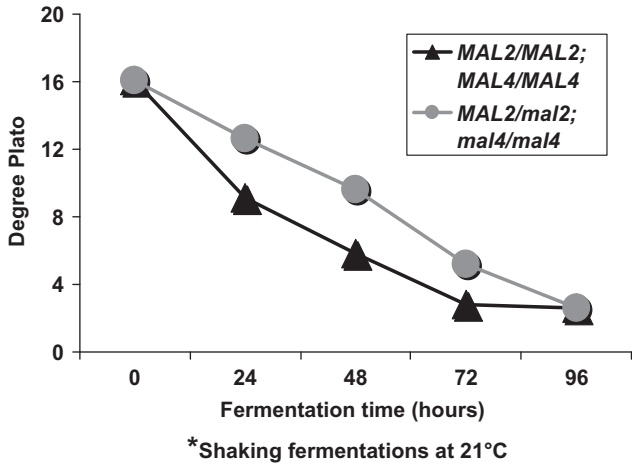


FIGURE 7.19 Fermentation profile of a 16°Plato wort with a diploid yeast strain containing multiple maltose (*MAL*) genes. *(From Stewart, 2006.)*

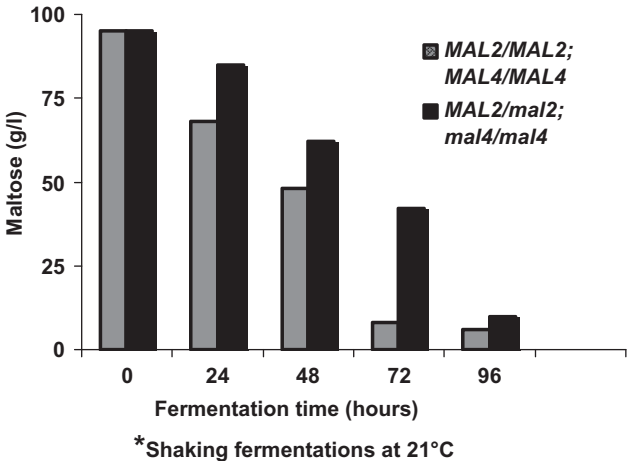


FIGURE 7.20 Uptake of maltose from 16°Plato wort by a diploid yeast strain containing multiple (*MAL*) genes. *(From Stewart, 2006.)*

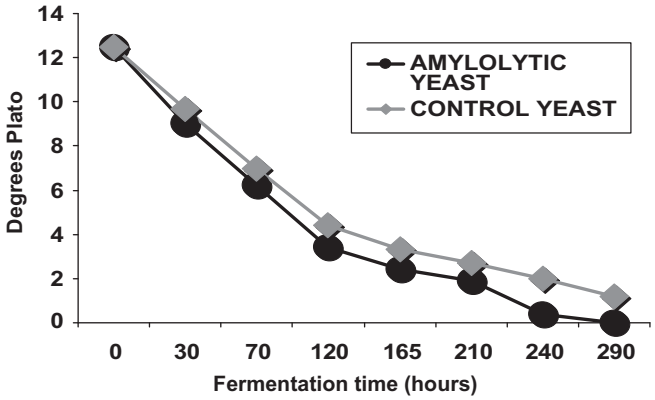


FIGURE 7.21 Effect of glucoamylase in a brewing strain during wort fermentation. *(From Erratt and Stewart, 1978.)*

D. Wort Free Amino Nitrogen

The complex composition of wort has already been discussed, with this medium containing many important ingredients. As well as the sugar spectrum, the level of FAN is important. Wort FAN consists of three components: amino acids, small peptides, and ammonia. FAN is a general measure of a yeast culture's assimilable nitrogen. It is a good index for yeast growth and therefore fermentation efficiency and sugar uptake (Inoue, 1992; Inoue and Kashihara, 1995). Wort FAN is essential for the formation of new yeast amino acids, the synthesis of new structural

FIGURE 7.22 Production of glucose in pasteurized beer during storage at 21°C. *(From Erratt and Stewart, 1978.)*

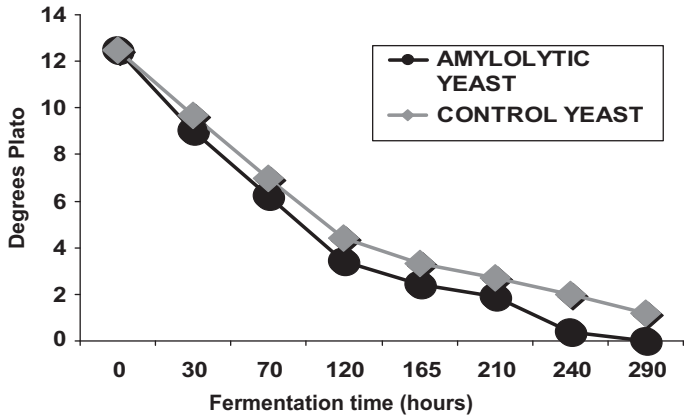


FIGURE 7.23 Brewing Research Foundation International: Nutfield Lyte. *(Source: author's own photograph.)*

and enzymatic proteins, cell proliferation, and cell viability and vitality. Lastly, FAN levels have a direct influence on flavor compounds in beer (for example, higher alcohols and esters).

There are differences between lager and ale yeast strains with respect to wort assimilable nitrogen uptake characteristics (O'Connor-Cox and Ingledew, 1989). Nevertheless, with all brewing strains the amount of wort FAN content required by yeast under normal brewery fermentation is directly proportional to yeast growth and also affects certain aspects of beer maturation, for example diacetyl management (Barton and Slaughter, 1992) (details described in the next subsection). There has been considerable polemic regarding the minimal FAN required to achieve satisfactory yeast growth and fermentation performance in conventional gravity (10−12°Plato) wort and it is regarded to be 130 mg FAN/l. For rapid attenuation of high-gravity wort (> 16°Plato), increased levels of FAN are required (Casey *et al.*, 1984). However, optimum wort FAN levels differ from fermentation to fermentation and from yeast strain to yeast strain. Furthermore, the optimum FAN values change with different wort sugar levels and type (Lekkas *et al.*, 2005).

During the 1960s, Margaret Jones and John Pierce, working in the Research Department of the Guinness Brewery in Park Royal, London, conducted notable studies on nitrogen metabolism during brewing, mashing, and fermentation. They reported that the absorption and utilization of exogenous nitrogenous wort compounds and their synthesis intracellularly are controlled by three main factors: (1) the total wort concentration of assimilable nitrogen; (2) the concentration of individual nitrogenous compounds and their ratio; and (3) the competitive inhibition of the uptake of these components (mainly amino acids) via various permease systems (Jones and Pierce, 1964).

Jones and Pierce (1964) established a unique classification of amino acids according to their rates of consumption during brewing wort fermentation (Table 7.9). There are four groups of amino acids. Three groups of wort amino acids are taken up at different stages of fermentation and the fourth consists of only one amino acid, proline (the

TABLE 7.9 Order of Wort Amino Acids and Ammonia Uptake During Fermentation

Group A	Group B	Group C	Group D
Fast absorption	*Intermediate absorption*	*Slow absorption*	*Little or no absorption*
Glutamic acid	Valine	Glycine	Proline
Aspartic acid	Methionine	Phenylalanine	
Asparagine	Leucine	Tyrosine	
Glutamine	Isoleucine	Tryptophan	
Serine	Histidine	Alanine	
Threonine		Ammonia	
Lysine			
Arginine			

From Jones and Pierce (1964).

largest concentration amino acid in wort), which is not taken up during brewing fermentations (Figure 7.24). This is because of the anaerobic conditions that prevail late in the fermentation. When this classification was developed, the methodology used (liquid chromatography for measuring individual amino acids, etc.) was iconic. Similar measurements today use automated computerized high-performance liquid chromatography (HPLC), and it is difficult to envisage the challenges that were overcome 50 years ago. The Jones and Pierce (1964) amino acid classification is the basis of today's understanding of the relative importance of individual wort amino acids during fermentation and manipulating wort nitrogen levels by the addition of yeast extract or specific amino acids during high-gravity brewing. However, this assimilation pattern is often specific to the conditions employed. The nutritional preferences of the yeast strain are perhaps the most significant. Because of the differences in malting barley varieties, brewing conditions, and yeast strains used worldwide in the brewing industry, a more detailed review is desirable.

Many studies (Lekkas *et al.*, 2007) have examined the absorption and utilization of wort amino acids and we now have a clear idea of their role during fermentation. However, approximately 30% of incorporated nitrogen compounds come from sources other than amino acids. Although the utilization of small peptides by brewing yeasts was confirmed before the 1950s, an understanding of their role in yeast nitrogen requirements is still limited. Small peptides can be used as nutritional sources of amino acids as carbon or nitrogen sources and precursors of cell wall peptides during yeast growth, although growth is much slower when they are the sole nitrogen source (Ingledew and Patterson, 1999). Polypeptides are also used as a substrate because yeasts can generate proteolytic enzymes extracellularly to provide additional assimilable nitrogen to the cells.

Most brewing yeast strains transport peptides with no more than three amino acid residues but this limit is strain dependent (Marder *et al.*, 1977; Nisbet and Payne, 1979). Nevertheless, small wort peptides are an important source

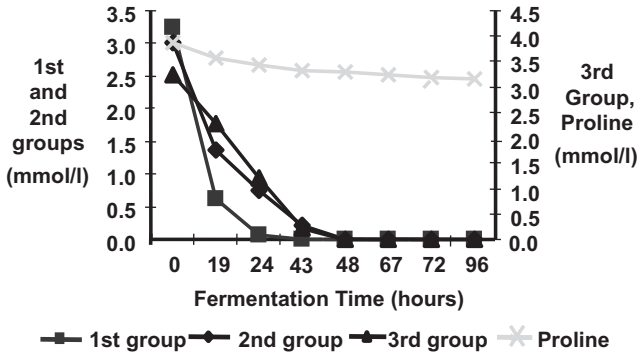


FIGURE 7.24 **Amino acid absorption pattern during wort fermentation.** *(From Lekkas* et al.*, 2007.)*

of assimilable nitrogen and 20−40% of wort oligopeptides are used during fermentation. In a similar way to single amino acids, peptides probably contribute to beer character and flavor. Wort peptides are taken up in specific order dependent on the amino acid composition. The determination of small (two and three amino acid units) peptides has been an impediment to this area of research. Recently, a method to measure these small oligopeptides has been developed (Lekkas *et al.*, 2009). In brief, the sample is deproteinized, the supernatant ultrafiltered through a membrane with a 500 Da exclusion limit, the filtrate is acid and alkaline hydrolyzed, and the hydrolysate subjected to HPLC analysis.

During fermentation, the yeast secretes a number of proteinases into the fermented wort (mainly proteinase A). Therefore, hydrolysis of medium-sized peptides occurs to smaller peptides, which can be taken up by yeast, and this continues throughout fermentation (Lekkas *et al.*, 2007). This fact highlights a difference between the uptake of wort sugars and FAN during brewing. The spectrum of wort sugars at the beginning of fermentation is fixed because after wort boiling, all malt amylases have been inactivated, whereas because of proteinase secretion by yeast, the spectrum of nitrogen compounds is dynamic. It is worthy of note that stress effects on yeast can increase proteinase secretion. For example, this occurs to a greater extent during the fermentation of high-gravity worts (Cooper *et al.*, 2000).

E. Wort Fermentation

As previously discussed, wort is a very complex medium and fermentation occurs largely in static systems (except for the agitation engendered as a result of carbon dioxide evolution as part of the glycolytic pathway), usually in large cylindroconical vessels. The metabolism of wort sugars and FAN has already been discussed, but a plethora of other metabolic reactions also occurs; indeed, there are too many to discuss all of them in detail. Consequently, only three reactions will be considered: (1) yeast management, including the initial stages of fermentation, when the intracellular yeast glycogen is used in the presence of oxygen to synthesize unsaturated fatty acids (UFAs) and sterols, which are incorporated into the yeast membrane structure; (2) the later stages of fermentation, which overlap into maturation and involve the management of diacetyl and other vicinal diketones (VDK); and (3) the harvesting (cropping) of the yeast culture for reuse by flocculation and/or centrifugation.

Yeast management in the brewing context refers to yeast handling between fermentations and into a subsequent fermentation. The critical parameter for all stages of yeast management (cropping, storage, acid washing, propagation, and repitching) is to maintain the viability and vitality of cultures in order to ensure that when the yeast is pitched into wort the lag phase is kept to a minimum. At the beginning of fermentation, synthesis of UFAs and sterols, which are essential for cell membrane components, occurs at the expense of glycogen (which is the yeast culture's intracellular storage carbohydrate) (Figure 7.25) (Quain *et al.*, 1981). This must occur to ensure a normal growth pattern of the yeast population during the wort fermentation process. For many years it has been known that yeast cells are unable to synthesize UFAs and sterols under strictly anaerobic conditions (Andreason and Stier, 1953). Consequently, oxygen (by aeration or, increasingly, from the use of gaseous sterile oxygen) is supplied to the wort in the initial stages of fermentation and has been the subject of considerable polemic for many years (Kirsop, 1974). Indeed, with the advent of high-gravity brewing, the concentrated medium requires increasing concentrations of dissolved oxygen at the beginning of fermentation.

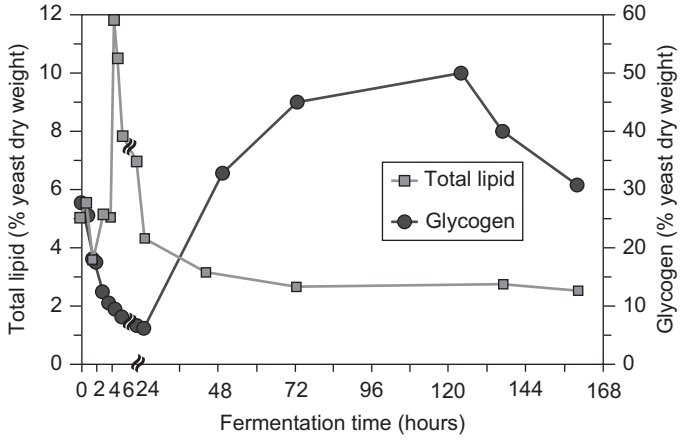


FIGURE 7.25 Intracellular concentration of glycogen and lipids in a lager yeast strain during fermentation of a 15°Plato wort. *(From Quain* et al.*, 1981.)*

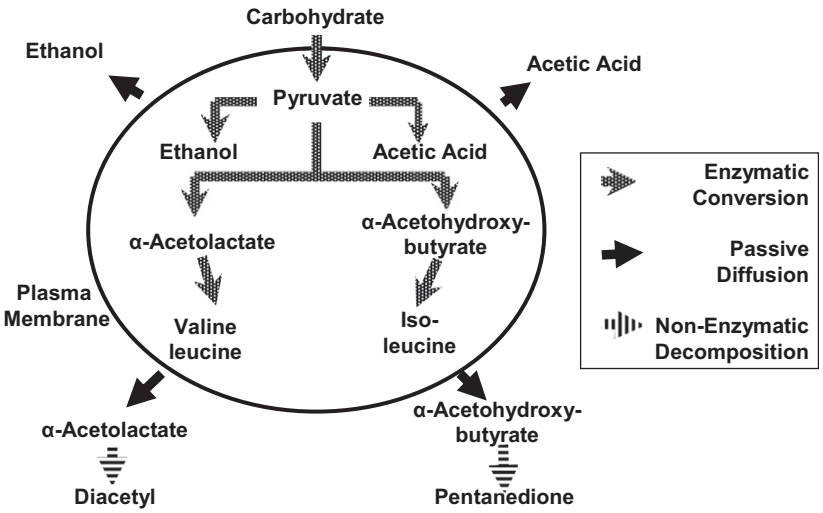


FIGURE 7.26 Fermentation of diacetyl and 2,3-pentanedione as by-products of pathways leading to the formation of valine and isoleucine. *(From Wainwright, 1973.)*

The VDKs diacetyl (2,3-butanedione) and 2,3-pentanedione impart an unwanted butterscotch or stale milk aroma to beer. Quantitatively, diacetyl is the most important since its flavor threshold is approximately 0.1 mg/l and is 10-fold lower than that of 2,3-pentanedione. The organoleptic properties of vicinal diketones contribute to the overall palate and aroma of some ales but in most lagers they impart an undesirable character. An exception to this is that in some lagers from the Czech Republic, VDKs can occur above threshold. A critical aspect of the management of lager fermentation and subsequent processing (maturation, aging, or lagering) is to ensure that the mature beer contains concentrations of VDKs lower than their flavor threshold.

Both VDKs arise in beer as by-products of the pathways leading to the formation of valine and isoleucine (Wainwright, 1973). The α-acetohydroxy acids, which are intermediates in these biosyntheses, are in part excreted into the fermenting medium. Here, they undergo spontaneous oxidative decarboxylation (Figure 7.26), giving rise to VDKs. Diacetyl is subsequently taken up by the yeast in suspension and reduced to acetoin and ultimately 2,3-butanediol (Figure 7.27) (Inoue, 1992). The flavor threshold concentration of the diols is relatively high and, therefore, the final reductive stages of VDK metabolism are critical in order to obtain a beer with acceptable organoleptic properties.

The reduction of VDKs in the later stages of fermentation and during maturation requires the presence of adequate yeast in suspension in the fermenting wort. Thus, where the yeast is particularly flocculent (this phenomenon will be discussed next), premature separation will be reflected by low rates of diacetyl reduction and potentially elevated levels in finished beer. Diacetyl removal is also affected by the physiological condition of the yeast. When the

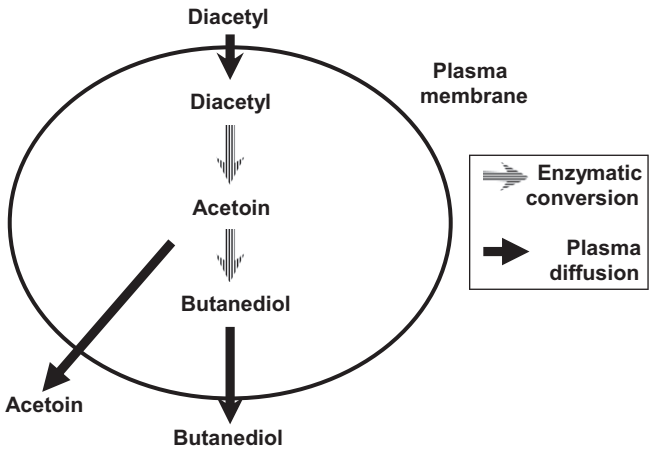


FIGURE 7.27 Reduction of diacetyl to acetoin and 2,3-butanediol. *(From Wainwright, 1973.)*

pitching yeast is in poor condition, such that the primary fermentation performance is suboptimal, the yeast present during the later stages will be stressed and the period of diacetyl reduction will be prolonged.

The flocculating properties of brewer's yeast strains have already been discussed and flocculation is one of the major factors when considering important characteristics during brewing fermentations (Stewart, 1973). Flocculation has many definitions (Stewart *et al.*, 1974). However, one that has been used for many years is 'the phenomenon wherein yeast cells adhere in clumps and either sediment from the medium in which they are suspended or rise to the medium's surface' (Stewart *et al.*, 1974). This definition excludes forms of 'clumpy growth' and 'chain formation' (Stewart *et al.*, 1973, 1974), which will not be discussed further.

The importance of flocculation to crop a yeast culture at the end of primary fermentation, so that it can be reused in a subsequent fermentation, cannot be overstated.

As flocculation is a cell surface phenomenon, the cell wall structure of the culture is critical. Therefore, appropriate findings on cell wall structure will be discussed. Genetic studies on yeast flocculation began over 50 years ago (Thorne, 1951) and Gilliland (1951) confirmed that this phenomenon was an inherited characteristic with flocculence being dominant over non-flocculence. The first flocculation gene (*FLO* gene) to be studied in detail was *FLO1*. Using traditional gene mapping techniques (mating, sporulation, micromanipulation, tetrad analysis, etc.) it has been shown that *FLO1* is located on chromosome I, 33 cM from the centromere on the right-hand side of the chromosome (Russell and Stewart, 1980a).

Novel genetic techniques have been developed, the principle of which is the sequencing of the *Saccharomyces* genome (Goffeau *et al.*, 1996). This sequenced laboratory strain contains five *FLO* genes, four located near the chromosome telomeres *FLO1*, *FLO5*, *FLO9*, and *FLO10*, and one neither at the centromere nor at the telomeres, *FLO11* (Querol and Bond, 2009). These genes encode lectin-like proteins which are also known as adhesins, zymolectins, or flocculins. The widely accepted model for yeast flocculation describes it as the result of the interaction between adhesins and mannans, polysaccharides built up of mannose residues, present on mannoproteins in the cell wall (Klis *et al.*, 2006). In most laboratory strains (haploid and diploid), added mannose will block adhesin-binding sites and thus inhibit flocculation by preventing the adhesions binding the mannose present on neighboring cells (Stratford, 1989). A similar adhesion of considerable industrial importance is responsible for the mannose-, glucose-, and maltose-sensitive 'new flo'-type of lager yeast strains (Teunissen and Steensma, 1995). In this case, competitive binding of such carbohydrates by this adhesion takes place and ensures that flocculation occurs only at the appropriate stage in the wort fermentation; namely, when all fermentable carbohydrates have been depleted and, hopefully, when VDK levels (particularly diacetyl) are under control.

It has been reported that yeast strains that exhibit ideal properties on day one of a fermentation can rapidly change and flocculate too early, or late flocculation onset occurs, or they may even lose the ability to flocculate altogether. A plethora of studies has described the presence of mutated sequences in the *FLO1* gene (Ogata *et al.*, 2008). Frequent intragenic recombination events will typically result in the net loss or gain of tandem repeat units. Expansion of the *FLO1* tandem repeat domain size results in stronger flocculation.

The genetic variability of flocculation genes has important consequences for studies and applications targeting these genes in brewing and distilling strains which have unknown genomes because of their polyploid and aneuploid characteristics. It has been revealed that there is considerable genetic variability in the chromosomal regions where flocculation genes are located. In future, knowledge of yeast flocculation mechanisms and control, particularly in the context of the brewing process, research, and industrial application, should be adapted to the flocculation gene families present in brewing yeast strains.

The other way to crop yeast for reuse and to clarify primary fermented wort is with the use of a disk stack centrifuge. As this process cannot be regarded as a biochemical process, it is beyond the scope of this chapter. The use of centrifuges in brewing has recently been reviewed (Chlup and Stewart, 2011). However, when a yeast is passed through a centrifuge it experiences mechanical and hydrodynamic shear stresses. This shear stress can cause a decrease in cell viability and flocculation, cell wall damage, increased extracellular proteinase A levels, hazier beers, and reduced foam stability.

V. EXOENZYMES IN BREWING

This chapter has endeavored to discuss many (but not all) of the important endoenzymes involved in the brewing process. As already described here, several enzymes of microbiological origin (exoenzymes) are being applied to the brewing process, many of which have been developed by genetic manipulation. The use of exoenzymes can be considered according to their use in the brewing process: adjunct liquefaction, malt and cereal enhancement

(high-adjunct brewing), smooth brewing operations, attenuation control, maturation, stability enhancement, troubleshooting, and innovations. Many exoenzymes are used in brewing as troubleshooting palliatives to try to overcome problems. It is not the intention here to discuss in detail each commercially available enzyme, because of their proprietary characteristics.

A. Adjunct

Adjunct (unmalted starch) is usually converted to fermentable sugars using enzymes from malt. However, the use of bacterial α-amylase with high heat stability instead of malt can offer the brewer the possibility of a simpler and less expensive process. There are many brewers who maintain that with high-quality malt containing a good enzyme level, with high diastatic power (DP), it does not matter that some activity is lost during the adjunct liquefaction process at 100°C. When using other malted cereals such as sorghum (or as an adjunct), where the DP is considerably less than barley malt, high dosages of α-amylase and pentosanase can be used as exoenzymes.

High-adjunct brewing requires separate cooking and liquefaction stages and the proportion of the malt in the grist may not be high enough to provide sufficient amylase activity for efficient starch conversion to fermentable sugars. This problem can be overcome by adding a saccharifying fungal or bacterial α-amylase either to the mash tun or at the start of fermentation. In addition, when small malt proportions are used, there is a possibility that insufficient FAN will be dissolved in the wort to provide good yeast nutrition. To overcome this problem, a protease can be used to enhance the wort FAN level, assuming there is sufficient protein to be hydrolyzed.

B. Barley Brewing

Barley brewing is the ultimate in high-adjunct brewing without any barley malt being used. This process has been investigated for its economic advantages. In addition, a ban on the import of barley malt was introduced in Nigeria to conserve valuable foreign currency. This ban forced Nigerian breweries to develop methods for brewing lager beer entirely from locally grown raw materials such as sorghum, maize (corn), and sorghum malt, although very little of this malt was available at the time. Brewing with this type of raw material has several problems, such as a lack of enzymes from malt, low nitrogen with the use of proteases, wort separation (enhanced with a mash filter), and gelatinization temperature, which involves the need to cook large proportions of the grist at temperatures much higher than the optimum of the available saccharification enzymes. One way to solve this problem is to use very low water-to-grist ratios for the cooker mashes (thick mashes) and to use low-temperature water and very high ratios of water-to-grist (thin mashes) for mashing the part of the grist that does not need cooking.

VI. WORT SEPARATION AND BEER FILTRATION

Wort separation and beer filtration are two common bottlenecks in the brewing process. Poor lautering not only causes a loss in production capacity, but can also lead to loss of extract yield. Furthermore, a slow lautering process negatively affects the quality of the wort, which may lead to problems with beer filtration, flavor, and stability.

A very important factor influencing filtration rates is wort viscosity. A thorough breakdown of β-glucans during mashing is one method leading to fast wort separation. Undegraded, dissolved β-glucans lead to worts with high viscosity, resulting in slower diffusion through the spent grains and less efficient extraction of fermentable sugars. Undegraded wort β-glucans that are carried into the fermenter will also negatively influence beer filtration, reduce filter capacity, and increase the consumption of filter aids (kieselgur or diatomaceous earth). Given the raw material quality and composition, as well as milling procedures, the solution to these problems is enhanced mashing. Sufficient levels of β-glucanase and pentosanase should be present in the mash, and mashing conditions should permit these enzymes to operate effectively. A wide range of β-glucanase/pentosanase preparations is available for reducing wort viscosity caused by glucans and pentosans. An unconventional method of speeding up the lautering process by reducing wort viscosity is to mash-off and lauter at 95°C. However, high mashing temperatures can negatively influence beer flavor and stability.

The availability of industrially produced amylases provides the brewer with a means to control the degree of fermentation. A wort produced under normal brewing conditions will generally give an apparent attenuation of 80−85% and approximately 25% of residual extract will be present in the beer. As already discussed, this residual extract, mainly non-fermentable short-chain dextrin material, contributes to beer mouth-feel and its calorific value (Brenner, 1980). Sometimes the residual extract specifications are not achieved because of malt quality deficiencies and/or mashing

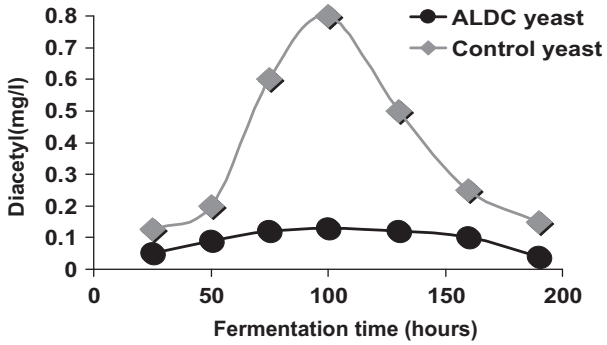


FIGURE 7.28 Effect of α-acetolactate decarboxylase (ALDC) expression in a brewing yeast strain on diacetyl metabolism during wort fermentation. *(Graphs plotted from data by Yamano* et al.*, 1994.)*

process problems, so corrections have to be made. Alas, where non-traditional raw materials are used, there may also be a need to use external saccharifying amylases to achieve the required degree of fermentation. Another possibility is to create new products by increasing the degree of fermentation even further by reducing the residual unfermentable dextrins; light (lite) and dry beers fall into this category. The goal might be to make small adjustments or to completely remove all of the residual dextrins, resulting in what is known as a super-attenuated beer. Such a beer will have fewer calories and less mouth-feel than a beer of normal attenuation (assuming a similar alcohol content).

Traditional brewing methods are available to achieve these adjustments, usually by modifying the mashing regime and using high DP malts. However, a range of exoenzymes exists for the same purpose and includes a number of saccharifying enzymes such as α-amylase, glucoamylase, and pullulanase (debranching enzyme) that are useful, practical tools when the attenuation needs to be adjusted for one reason or another.

The management of diacetyl and other VDKs during the later stages of fermentation and during maturation has already been discussed in this chapter. An alternative way to manage diacetyl and related compounds is to use the enzyme α-acetolactate decarboxylase (ALDC). This enzyme can decarboxylate α-acetolactate into the flavor-inactive acetoin and bypasses the formation of diacetyl. ALDC is obtained from the generally regarded as safe (GRAS) Gram-negative microorganism *Acetobacter aceti*, which is also used to metabolize ethanol into acetic acid during vinegar manufacture. The availability of an ALDC (marketed under the tradename Maturex®) now offers the brewer a possibility of bypassing the conversion of α-acetolactate to diacetyl, which is a slow reaction. However, ALDC must be added to the wort at the start of fermentation.

A novel alternative approach was to clone the gene for ALDC into a brewing strain. The ALDC gene was obtained from a strain of *A. aceti*. Wort fermentation trials with the ALDC yeast were conducted and compared to the uncloned strain as control. The diacetyl production and reduction profile were profoundly different compared to the uncloned control culture (Yamano *et al.*, 1994) (Figure 7.28). Because of the presence of ALDC, the α-acetolactic acid was not spontaneously converted to diacetyl, but to acetoin instead. The overall fermentation performance of some cloned yeast strains can be adversely affected compared to the uncloned control strain. This was not the situation with the ALDC yeast (Figure 7.29).

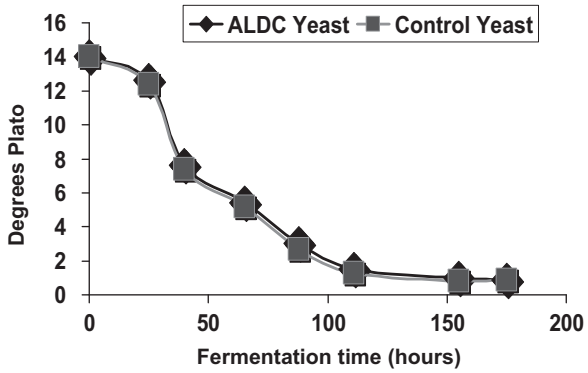


FIGURE 7.29 Effect of α-acetolactate decarboxylase (ALDC) expression in a brewing yeast strain on overall fermentation rate during wort fermentation. *(Graphs plotted from data by Yamano* et al.*, 1994.)*

Beer stability can be enhanced by the use of exoenzymes. This is particularly true of physical stability, also called haze formation. With a few notable exceptions, consumers prefer their beer to be bright and free of particles. Stored beer, particularly at room temperature, has the potential to produce haze and its brightness will be compromised. This haze consists of an association of small polymerized polyphenols and polypeptides which are high in the amino acid proline (Siebert and Lynn, 1997). This permanent haze does not redissolve even when the beer is warmed to 30°C or higher. The balance between flavenoid polyphenols and 'sensitive' proline-containing hydrophilic proteins largely dictates the physical (colloidal) stability of beer. Haze will not form, or its formation will be retarded, when either of these components is removed or the factors promoting the interaction are largely excluded. A number of procedures can be employed to retard and/or prevent haze formation:

- Prevent the formation of large amounts of complex protein degradation products during beer production, particularly mashing.
- Remove some of the polyphenols or the sensitive proteins during brewing with specific adsorbants (polyvinylpolypyrrolidone or silica gel).
- Store maturing beer cold (0°C or less) to precipitate haze precursors − this is a time consuming process.
- Store packaged beer cold (2−4°C) to retard haze formation.
- Hydrolyze, with exoenzymes, the complex sensitive polypeptides. Broad-spectrum proteases, e.g. papain (from papaya), ficin (from figs), and biomelain (from pineapple) have been used for many years to enhance the physical stability of beer. However, these enzymes are non-specific; therefore, as well as hydrolyzing sensitive polypeptides, they will hydrolyze the hydrophobic polypeptides that stabilize beer foam, and foam-enhancing agents, such as polyglycol alginate, have to be added to put a foam head on a glass of beer. Recently, a proline-specific endopeptidase has been developed. This enzyme specifically hydrolyzes sensitive polypeptides because of their enhanced proline content and promotes the physical stability of beer with no effect on foam stability (Lopez and Edens, 2005).

VII. CONCLUSIONS

Brewing is largely, but not exclusively, a biochemical/enzymatic process. However, the current process also involves plant science, microbiology (closely related to biochemistry), chemistry, physics, engineering, process control, and flavor assessment. It is normally a batch multienzyme process that involves a number of clearly defined, but overlapping, stages. The modern-day brewing process has two objectives. The first objective is efficiency, making maximum use of the available overheads, both fixed and variable. The second objective is to produce high-quality drinkable beers that possess enhanced stability. Brewing is a traditional process with a long history that focuses upon four major raw materials: barley malt; hops; water; and yeast. The advent of high-quality microbial enzymes, some of which have been developed with the use of genetic manipulation, has permitted the use of non-malt enzymes in the brewing process that will, under certain circumstances, assist the efficiency of the process and the quality and stability of the product. However, brewers are still reluctant to use such enzymes.

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