Mashing

6

6.1 Purpose of Mashing

Mashing is necessary as a step in the brewing process. Essentially, it is a continuation of the germination process begun in the malt house. However, the difference between germination and mashing is that the barley seed does not continue its growth after being kilned. In mashing, the enzymes inside the barley seed become reactivated. Once hydrated and at the correct temperature, the enzymes go to work and convert the starch into fermentable and non-fermentable sugars. They also decompose the proteins and other biological structures holding the starch in the endosperm and release it into the water. They convert the proteins into smaller pieces and individual amino acids, and also help lower the pH of the overall system.

The fermentable sugars are very necessary in the next step of the brewing process. These sugars include maltose, glucose, maltotriose, and a host of others that can be consumed by yeast during fermentation. While fermentable sugars are the primary product of the mashing process, mashing also creates non-fermentable sugars. These sugars are not consumed by yeast during fermentation and remain essentially unchanged by the end of the entire process. In other words, they remain in the beer after fermentation to lend a sweet taste.

Mashing also reduces the size of some of the proteins that are extracted from the malt. In some cases, the individual amino acids are cleaved from the proteins. Many of these are essential as nutrients for the yeast during fermentation. The amount of protein degradation during mashing has a direct impact on the mouthfeel and the properties of the head of the final beer. As we will uncover later, other enzymatic activities can even result in a lowering of the pH of the wort to the perfect level for other enzymatic activity.

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6.2 Equipment Used in Mashing

There are many different types of mashing vessels available in the market. In the microbrewery, cost and efficiency often result in the use of a mash tun that can double as a lauter tun (see the next chapter for more information on this step in the brewing process). In the large-scale brewery, when performing mashes on large batches, or when using malt that has been milled using a hammer mill, the use of a separate vessel for separating the spent grains from the wort is necessary.

Let us take a look at the process in a stepwise fashion. The first process in the mashing process is to combine the grist with hot liquor. First, we add *foundation liquor* to the mash mixer or mash tun. This hot liquor serves multiple purposes and is a vital step in the process. It preheats the mash vessel and provides a buffer for the grist that will fall into the vessel. Without the water at the bottom of the vessel, the grist could fall with such force that it would be driven into the holes of a false bottom or even further break the grist or the husks into smaller pieces. Finally, the foundation liquor helps reduce the amount of air that is mixed into the mash and results in a reduction of oxidation during mashing.

Once the foundation liquor is in the vessel, we can add the *grist* through the top of the vessel either directly into the vessel or after a premash mixing with additional hot liquor. The Steel's Masher, a common premash mixer, was developed in England in 1853. It has not changed much since then because of how beautifully it mixes the grist and hot liquor. This device, see Fig. 6.1, admits dry grist through a grist case and into a horizontal arm. Hot liquor is injected into the grist at this point. Then, an auger moves the grist/water mixture and mixes it before directing it into the mash vessel. Use of a Steel's Masher gives the brewer control of the temperature of the initial mash-in and mixes the mash sufficiently such that it does not have to be mixed much, if at all, during an infusion mash.

Another common premash mixer that we find in the brewery is the vortex masher. This device can be used with dry and wet milled grist. The grist is added to a funnel at the top of the mash vessel while the hot liquor is also added. Some designs allow the hot liquor to be sprayed into the grist to aid in the reduction of dust. Other designs simply add the grist to a stream of hot liquor. The vortex masher adds the two streams together in such a way that they swirl around each other as they enter the mash vessel. This mixing constitutes the start of mashing, known as

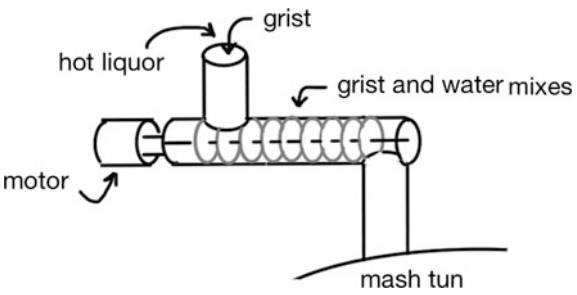


Fig. 6.1 Steel's Masher

the mash-in. The design of the masher is such that the grist is fully hydrated by the time it enters the mash tun.

Alternative premash mixers have also been developed. In some cases, these involve adding hot liquor to the grist and using a positive displacement pump to transfer the wet slurry into the mash tun via the bottom of the vessel. And, as we would expect, there are some breweries that simply add the grist directly to the mash tun containing the appropriate amount of hot liquor.

In the homebrewing world, this is the process that takes place. The mash tun (for the homebrewer, this is often a cooler) is filled with hot liquor whose temperature is just a little warmer than the desired mash temperature. Then, the grist is added and the mixture is stirred with a long paddle or spoon. Stirring is continued until all of the grist has been wetted (the dough balls are broken up). In fact, the authors have even seen some homebrew setups where the grist is placed in the cooler first, and then the hot liquor is added with a LOT of stirring.

While the processes at the homebrewery level are not ideal, they work. When translated to the larger scales of microbrewing, they can still work—but cost a lot in terms of time and elbow grease. For the small, start-up microbrewery, the addition of dry grist to a mash tun full of hot liquor can be a way to complete the mash-in. It is not efficient, but it works and is a little less expensive than the use of a premash mixer. The most effective part of the simpler process is that a brewer can easily get the correct ratio of water and grist during the mash. Compared to the use of a vortex mixer, the rate of addition of water versus the rate of addition of grist must be closely monitored to arrive at the correct mass of both in the final mash.

CHECKPOINT 6.1
What is the purpose of the Steel's Masher? What benefit would it or another premash mixer have on the outcome of the mashing step in brewing?

6.2.1 Cereal Cookers

A cereal cooker is a separate mash vessel needed when an unmodified adjunct is added to the mash. Unmodified cereals that are commonly used in brewing include corn and rice. Unmalted barley, oats, wheat, and rye also find their way into the brewing recipe. Initially, the use of unmodified grains was common as a way to save expense in the manufacture of beer. Today, the cost is a small part; the main reason for the use of unmalted cereals is that they are an integral part of the recipe. In other words, the use of adjuncts may be desired for the final flavor or the beer.

If these adjuncts are used, we have to "modify" them so that their starch is available for fermentation. That modification is essentially the same thing that happens when barley is malted, with one large exception. None of the enzymes will be produced or survive the process. To do this, the brewer mills the cereal to break

open the grains and then adds them to the cereal cooker. The addition occurs in the same ways that malt is added to the mash vessel. The slurry of cereal grist and hot liquor is stirred while the temperature of the vessel increases. The final temperature of the mixture is based upon the type of cereal used (see Table 6.1). This temperature allows the starch in the cereal to become available for enzymatic action during the mash. Stirring aids in this process and also keeps the mixture from scorching on the side of the heated vessel. It is important to note that while the process makes starch available for enzyme action, no enzymes are activated in the cereal cooker.

Cereal cookers are required for unmodified adjuncts because those grains have not been malted. For example, if we add a handful of milled barley to hot water and waited a few minutes, we would easily see that this is true. The "barley tea" we just made would not be sweet. The lack of sweetness in the water indicates that none of the starch has been converted into sugars. Because the cereals have not been malted, none of the enzymes needed to convert the starch into fermentable sugars are present. In other words, cereal added to hot liquor will not mash. However, if the starch is made available by breaking down the cell walls and protein coats, the resulting slurry can be added to an existing mash. That slurry would be rich in starch and available to be mashed. The enzymes in the existing mash made with fully or partially modified grains can convert all of the starch from the cereals into fermentable (and non-fermentable) sugars.

Once the cereal has maintained its gelatinization temperature for a specified amount of time (usually 20 min or so), it is pumped while still hot into the mash mixer with the malted grist. The malted grains and the cereal grains are mixed to make sure that the enzymes have access to all of the starch in the slurry. This mixing results in raising the temperature of the mash overall because the slurry from the cereal mixture has a higher temperature. A typical temperature profile for the result is shown in Fig. 6.2. Note that the result of adding both the gelatinized grits and the malted mash results in the final saccharification temperature where the starches are converted to sugars. While the heated cereals could be added with the initial mash-in and then treated just as the mash is treated, the starch in the cereals would not be available for the enzymes in the mash. And if that was done, we would only have sugars that result from the malted grains, plus a bunch of flavor

Table 6.1 Gelatinization temperatures of unmodified cereals

Cereal	Gelatinization temperature
Barley	52–59 °C (126–138 °F)
Corn	62–72 °C (144–162 °F)
Oats	53–59 °C (127–138 °F)
Potato	56–71 °C (133–160 °F)
Rice	68–77 °C (154–171 °F)
Rye	57–70 °C (135–158 °F)
Sorghum	68–75 °C (154–167 °F)
Wheat	58–63 °C (136–147 °F)

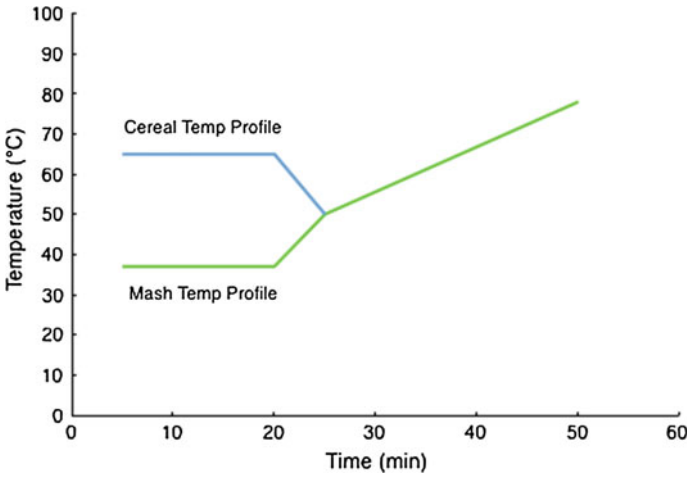


Fig. 6.2 Gelatinized cereal addition to mash raises temperature of the mash. Note that this mash profile holds at 37 °C for 20 min, then the temperature is raised to 50 °C by the addition of the gelatinized cereal, and then ramped up to 78 °C over the next ~25 min. This ramp allows the brewer to hit each of the mash rests and provides the flavor profile that the brewer is looking for in this case

from a hot extraction of unmalted grains. In addition, adding them later in the mash steps saves energy associated with warming the overall mash.

6.2.2 Mash Mixer and Mash Kettles

The typical mash mixer, fitted with the Steel's Masher or vortex mixer, contains rotating paddles at the bottom to stir the mash, see Fig. 6.3. The slurry of grist and water is stirred during the entire mashing process to ensure an even temperature, an even distribution of all of the enzymes, and provides some protection from scorching the mash if the vessel is heated. Stirring must be carefully monitored during the mashing process, because the speed of stirring is directly related to the amount of shear stress transferred to the mash. A low shear rate, and a slower stirring speed, improves the quality of the finished beer. If the mash is stirred too violently, the high shear stress on the mash can result in lower enzymatic activity, smaller protein fragments, and fracturing of the grist that decreases the particle size (which may result in a stuck mash during the next step in the process).

Modern mash mixers are not symmetrical in their design. The offset stir paddles increase the mixing of the mash without increasing the shear stresses. The mixing blades are also positioned so that a space exists between the blade and the sides and bottom of the vessel. This space is necessary so that the grist is not further ground by the blades.

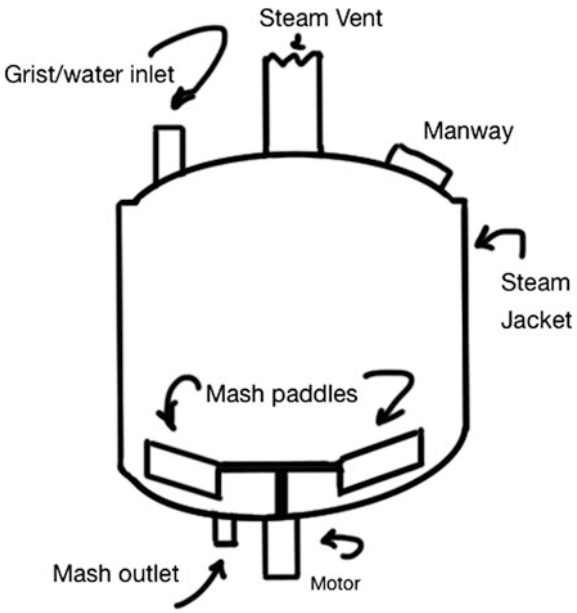


Fig. 6.3 Mash Mixer. Note the asymmetric *bottom* on the mixer that improves mixing without increasing shear forces. The vessel is also steam jacketed to allow temperatures to be maintained or adjusted to match the desired temperature. Not shown is the CIP system that is almost always included in the mixer

Steam jackets surround the sides of the modern mash mixer. Typically, the jackets provide different heating zones inside the mash vessel. In this way, the mash can be slowly or differentially heated. Alternatively, additional hot liquor or a gelatinized cereal can be added to adjust the temperature.

Mash kettles can be used in the mashing process and are required if the brewer's recipe requires a *decoction mash*. The mash kettle itself looks nearly identical to the cereal cooker. In a decoction mash, a portion of the mash (both liquid and grist) is pumped from the mash mixer into the mash kettle. Then, the temperature of the mash kettle is increased, typically to boiling. This allows Maillard reactions (the reaction of amino acids and sugars) to take place and increase the browning and caramel-like flavors. The heated and stirred mash is then transferred back into the mash mixer—the addition of which raises the temperature of the mash.

An example profile for a double-decoction mash using a mash kettle is shown in Fig. 6.4. In this particular mash, the temperature of the mash-in results in a temperature of 37 °C (the dough-in rest, see Sect. 6.4). The temperature is held constant for a while, and then approximately one-third of the entire mash is removed to the mash kettle. The removed mash is then heated to boiling and kept there for about 5 min. When the boiled mash is returned to the mash mixer, it raises the temperature of the entire mixture to 50 °C (the protein rest, see Sect. 6.4). The temperature of the mixture is maintained for about 10 min, and then another one-third of the entire mash is removed and heated to boiling in the mask kettle. Again, after returning this to the mash kettle, the temperature of the entire mixture is raised to 63 °C. This is the saccharification rest where the starches are converted

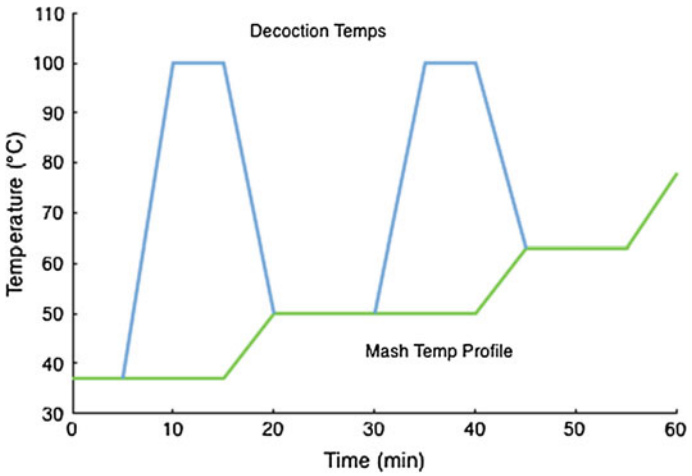


Fig. 6.4 One possible double-decoction temperature profile. Note that when some of the mash is withdrawn, boiled, and then added back into the mash, the temperature is raised to the next rest temperature. This example shows the decoction pulled from the dough-in temperature, returned to make the protein rest at 50 °C, pulled, and returned to give the saccharification rest. The entire mash is then raised in temperature to the mash-out temperature

into fermentable and unfermentable sugars. After 10 min, the entire mash temperature is raised to the mash-out temperature of 78 °C.

A more typical decoction is to withdraw one-third of the mash after the protein rest starts (50 °C) to raise the temperature of the entire mash to 63 °C. After a 20- to 30-min rest, the second decoction is used to bring the entire mash to the mash-out temperature.

Can we calculate the new temperature at each step when we do a decoction mash? In short, yes. Using the standard principles of heat transfer, the process is relatively straightforward. Because we know the temperatures that you want and the temperatures that you have, the calculation is most easily done by determining the amount of the mash to withdraw for the decoction. For example, assume that we start with a dough-in at 37 °C and want to raise the temperature of the mash to the protein-rest stage at 50 °C. We use the formula:

$$\frac{\text{Temp change of mash}}{\text{Temp change of decoction}} = \frac{50 - 37}{100 - 37} = 0.20$$

So, we would remove 20 % of the mash, decoct it, and then return it to the original mash. The temperature of the mash would then raise from 37 to 50 °C. The formula is very useful and works for any decoction (as long as the specific heats of the mash and the decoction do not change during the process).

Each time a portion of the mash is removed and heated to boiling, the enzymes become denatured at the elevated temperature. In fact, many of the proteins in the

boiling decoction are degraded. This results in thinning of the mash (making it easier to transfer) and destroys any further enzyme activity. However, since the boiled mash is returned to the unboiled portion, it mixes again with more enzymes. So, the overall end result is that the mash gets thinner, some of the proteins become degraded, and additional caramelization is added to the wort.

Because the mash is being transferred from one vessel to another, whether the mash kettle is used or not, a fairly thin mash is needed. Typically, the ratio of water to grist is 3–5 L/kg (~0.35–0.60 gal/lb). This means that temperature and pH control are very important to the brewer. The enzymes in this thin mash can easily be destroyed by small variations in the temperature or pH that deviate from the ideal. Thick mashes tend to buffer the enzymes from small changes in the temperature or pH because of the relatively large amount of hydrated grist to water ratio. As the amount of grist drops, its ability to buffer the changes in temperature and pH also drops, resulting in a loss of enzymatic activity in the mash.

CHECKPOINT 6.2
Figure 6.4 indicates one possible double-decoction profile. Draw the profile that would exist from the more "typical" double decoction outlined in this section.

6.2.3 Mash Tun

In some operations, the mash tun is the preferred, and only, vessel for performing the mash operation. The mash tun is an insulated or jacketed vessel that is operated similarly to the mash mixer. First, hot liquor is added to the vessel to prewarm it and provide protection to the grist as it is added. Addition of the grist can then occur via a premash mixer. Alternatively, in the most basic of mash tuns, the dry grist is added directly to a tun filled with hot liquor. In these cases, the hard work of the brewer to mix the grist and the hot liquor determines the quality of the mash. The end result in both the basic tun and that fitted with the latest gadgets is to end the mash-in with a temperature that starts the enzymatic processes.

Variability exists in mash tun design. As noted above, the simplest design is just an insulated vessel with a metal cover (that may or may not be used). More complex designs may include stirring paddles, mash rakes, or heating jackets; the simple vessel contains none of these, requiring the brewer to stir the mash with a handheld mash paddle to ensure even distribution of hot liquor and grist. Some designs even include a special rotating paddle that aids the removal of the spent grain from the vessel after mashing has finished.

The key in the use of the mash tun is that the vessel must be able to maintain the desired temperature. An even and steady temperature during the mash rest ensures the greatest efficiency in the process. While it is possible to raise the temperature of

the mash in the mash tun to different set temperatures, most uses of a mash tun involve a single temperature, known as a single infusion mash. The brewer selects the temperature of the single infusion mash to represent the flavor profile and alcohol content that they desire in the finished beer.

Most mash tuns are fitted with a false bottom, though the most basic vessels may have a network of perforated pipes that are placed in the bottom under the grist. The false bottom, or perforated pipe, allows the vessel to also serve as a lauter tun. The brewer could decide to pump the entire mash into a separate lauter tun, or when a false bottom is present, the wort can be drained from the spent grains.

6.2.4 Processes in Mashing

No matter which mashing vessel is used, the brewer takes great care to make sure that the temperature of the contents is monitored. The temperature is quite important in mashing as it determines the result of the wort. How does it do this? While hotter water can extract different components from the grain that cold water cannot, the temperatures are chosen to allow enzymes in the malt to do their job and convert starches into sugars.

If the mash is too hot, the resulting beer would be very thick in its mouthfeel, very sweet in taste, and have a very low alcohol content. If the mash is too cold, the opposite would happen; the resulting beer would be very thin and have a significant amount of alcohol, but very little sweetness. If the temperatures were even further away from the values that are needed in the mash, the resulting beer may have the flavor of tea, or taste very similar to the water that was initially added.

6.3 Enzymes and What They Are

When the 20 essential amino acids polymerize in a biological system, the arrangement and combinations are what scientists call proteins. The specific amino acids used, the order in which they are arranged, and the specific three-dimensional structure that the resulting polymer adopts determines the function of the protein. In some cases, the protein adopts a fibrous shape and may find its use in the structure of a biological system. Hair, for example, is made of a protein known as keratin. Keratin is a polymer of amino acids bonded together in a chain (the amino acid cysteine makes up about 14 % of the amino acids in human hairs). In other cases, the protein is more globular in nature. Myoglobin is an example of a globular protein containing over 150 amino acids. This polymer of amino acids holds a single iron ion that is used to bind to oxygen and store it near muscle cells that might need it later.

If the result is a globular protein, the molecule can have very specific properties. If one of those properties is to perform a reaction, we say that the arrangement of amino acids is actually an enzyme. Enzymes are biological polymers of amino acids

that have a globular shape and cause specific reactions to take place in the biological system. For example, lactase is an enzyme that can catalyze the rate of reaction of lactose into glucose and galactose. In mashing, the enzymes come from the malt used in the process. And like all enzymes, they conduct very specific reactions.

Unfortunately, enzymes are very fragile. They are easily degraded, first by unfolding and untwisting in a process called *denaturation*, and then by breaking back into their individual amino acids. Once denatured, an enzyme is unable to be "fixed." Heat is one of the culprits that can denature an enzyme. If the temperature is low, the enzyme has very limited ability to conduct its reactions. As the temperature increases, the enzyme's activity increases until it reaches the maximum activity at a particular temperature. Above that temperature, the enzyme begins to denature and the overall activity decreases. If the temperature is reduced after the enzyme is denatured, the enzyme is still unable to be repaired and the reactivity of the molecule is lost (see Fig. 6.5).

The alkalinity or acidity (the pH) of the solution with the enzyme is also important in the reactivity of the enzyme. If the pH is too low (the solution too acidic), the activity of the enzyme is lowered. If the pH is too high (the solution too alkaline), the activity of the enzyme is lowered. Only within a specific pH range is the enzyme active. While it is possible for the enzyme to denature when the solution is outside of the pH range, as long as the deviation from the pH range is not too great, the enzyme will not be denatured (Fig. 6.5).

Shear forces are the last of the damaging factors that can affect an enzyme's activity. A shear force occurs when one portion of the solution moves in an opposite direction from another portion of the solution. This can easily occur when a solution is stirred, mixed, or as we have seen, when the grist is dropped into the mash tun. The shear forces cause stress inside the molecules and can literally tear them apart. An enzyme can be torn apart into its original amino acids. Obviously, then, the enzyme is no longer capable of performing reactions.

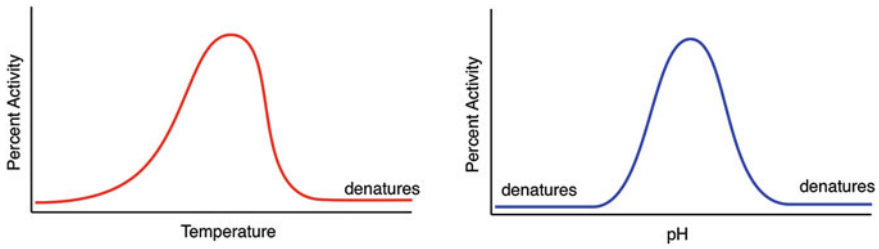


Fig. 6.5 Enzymes are active based on the pH and temperature of the solution. Note that enzymes do not denature below their optimum temperature, and that the pH must deviate a lot from the optimum before the enzyme denatures

When everything is right with the pH, temperature, and limited shear forces, the enzymes can do their reactions. Since every enzyme is a little different, each has a specific temperature and pH that describes its maximum activity. It is the job of the brewer to select the correct temperature and pH that allows the enzymes to do their job and to avoid high shear forces by stirring only as much as is absolutely necessary.

CHECKPOINT 6.3
In your own words, describe the term "shear."
Draw a picture of a mash tun and outline the differences it has with a mash mixer. What differences are there between a cereal cooker and a mash mixer?

6.4 Chemistry While Resting

As we have noted, the brewer selects the temperature of the mash to allow a particular enzyme to "turn on." The temperature is then held for a given amount of time to allow the enzyme to work. These hold times at specific temperatures are known as "rests." The enzymes involves at these particular rests perform specific chemical reactions. Because the enzymes are active over ranges of pH and temperature, many different enzymes might be active at any given pH and temperature.

In this section, we will explore some of the more important enzymes that are active during the mashing process. In order to get a handle on how some of these enzymes work, we will first uncover some of the chemistry associated with starch and related polymers of glucose. You may wish to refer back to Chap. 3 along the way.

6.4.1 Starch

As we learned in Chap. 3, glucose is an example of one of the carbohydrates. The carbohydrates themselves got their name because their chemical formula appears to be "hydrates" of carbon. For example, the formula for glucose is $C_6H_{12}O_6$ or $C_6(H_2O)_6$. Glucose is likely one of the most important carbohydrates because it is so prevalent in the world. For example, glucose is the monomer that makes up starch and cellulose. It is also found in barley as the monomer that makes up glucan (a structural component of the seeds). How does the same monomer make so many different polymers? If there were additional monomers included, we could assume that the main differences in the polymers were due the number and order in which

the monomers were attached. Interestingly though, starch and cellulose do not have other monomers in their structure. They differ only in the way in which the glucose molecule is attached to make the polymers.

Glucose itself is an interesting molecule. It contains a set of five different alcohol functional groups and one aldehyde functional group. In water, the molecule wraps around itself and one of the alcohol functional groups reacts with the aldehyde. The aldehyde functional group becomes known as the anomeric center. This gives rise to either a five-membered ring (known as glucofuranose) or a six-membered ring (known as glucopyranose) as shown in Fig. 6.6. To make things a little more complicated, as the alcohol group approaches the aldehyde group, it can either attack from the top or the bottom. The aldehyde functional group becomes known as the anomeric center. If the alcohol group attacks the aldehyde from the top, the resulting anomeric center ends up with an OH attached to it points down (known as the α-anomer). If it attacks from the bottom, the result is the β-anomer. Thus, in water, there are actually five different forms of glucose: open-chain glucose, α-glucofuranose, β-glucofuranose, α-glucopyranose, and β-glucopyranose).

Note that when we draw carbohydrates, we often use a combination of the line drawing process and simply just writing all of the atoms. If the molecule exists in a ring, the carbon atoms in the ring are omitted from the drawing to make it easier to see the entire structure. When we draw the molecule in open chain format, this can also be done, but for our purposes, the molecules are drawn showing all of the carbon atoms.

The presence of an OH group on the anomeric center signals to the brewer that the ring can open and close on its own. In other words, when the glucose molecule

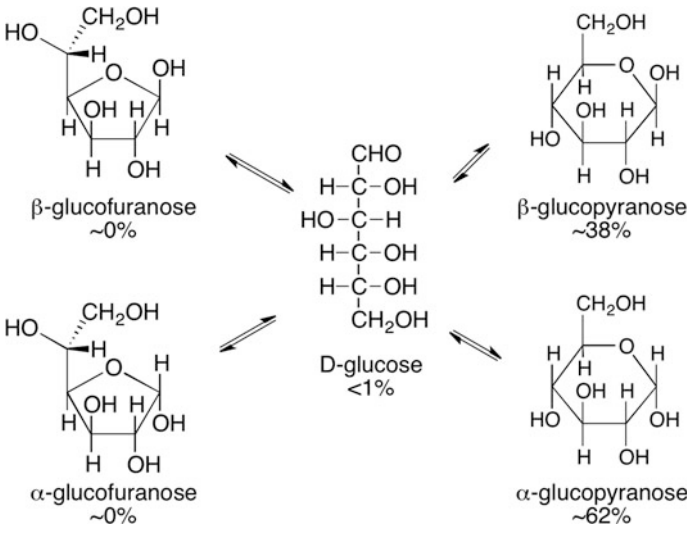


Fig. 6.6 The five forms of glucose found in water. Their relative percentages are <1% open-chain glucose, trace amounts of α-glucofuranose and β-glucofuranose, ~62% α-glucopyranose, and ~38% β-glucopyranose

bends over and closes to make a ring, it can also reverse the action and open back up to form the straight-chain form of glucose. As long as the OH group exists when the ring closes, the ring can reopen. Thus, the aldehyde group becomes masked and hidden when the ring is closed, but is easily observed when the ring reopens. Reduction in glucose is possible because the aldehyde functional group exists whether the ring is closed or not. We say that glucose is a *reducing sugar*.

As we noted in Chap. 3, glucose has a handedness. The common natural glucose found in nature has a mirror image of itself that is also known as glucose. That mirror image is completely unlike the glucose that we know. A designator is often used to distinguish between the two based upon the location of the alcohol functional group that is on the last chiral center farthest from the aldehyde. If the alcohol is on the right-hand side, the designator is "D" (from the Latin word "dexter"). On the left, we call the molecule "L" (from the Latin word "laevus") as shown in Fig. 6.7. By convention, the L and D are written as small caps. The naturally occurring form of all carbohydrates is the D-sugar; living things on this planet have evolved to exclusively prefer the use of the D-carbohydrates. While this does not mean that an occasional L-carbohydrate might be encountered from a natural source, it does mean that they are fairly rare. Therefore, the most common form of natural glucose in water is known as α-D-glucopyranose.

When two glucose molecules condense together to make a dimer, a new molecule is born. The dimer results from the reaction of an alcohol functional group on one of the glucoses reacting with the aldehyde position on another glucose molecule in exactly the same fashion as when glucose folds over onto itself to make the five- and six-membered rings in water. In Fig. 6.8, we note that there are two possible dimers that result. These two forms arise from the OH of one glucose monomer attacking either the top or the bottom of the aldehyde on the other monomer.

The two resulting dimers of glucose are not the same. Maltose is the sugar that we need for the yeast to consume during the fermentation step of the brewing

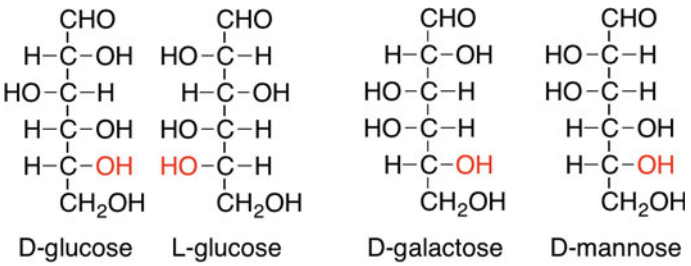


Fig. 6.7 D and L convention for carbohydrates. Note that the OH on the chiral center farthest from the aldehyde is on *the right-hand side* for "D" and on the *left-hand side* for "L." Also note that D-glucose and L-glucose are mirror images of each other that are not superimposable (they are enantiomers)

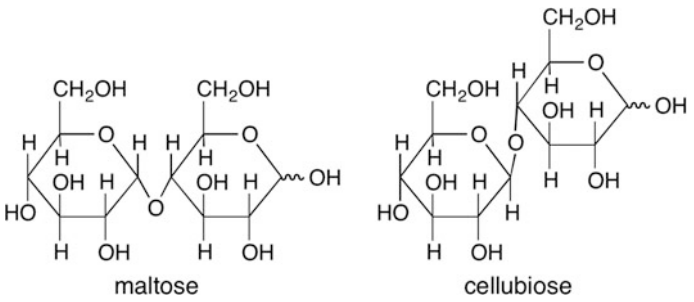


Fig. 6.8 Dimers of glucose. The major difference is the position of the oxygen attached to the anomeric carbon on the *left-hand molecule*. In maltose, that oxygen is down; in cellobiose, it is up. The wavy line on the anomeric carbon in the *right-hand* glucose molecule indicates that the OH can either be up or down

process. Cellobiose, on the other hand, is undigestible by yeast (and humans) and is not useful in brewing.

To distinguish between the possible dimers that can be made from the combination of carbohydrate monomers, we use a symbolism that represents the attachment. First, the position of the OH that is part of the aldehyde that was attacked is noted as either a or b. Then, in brackets, we write the position number of the first sugar and the position number of the second sugar monomer. For example, maltose has an α-[1 → 4] link. Note that the position of the OH on the aldehyde that was not attacked is not reported in this nomenclature. This is because the molecules are often in water and when they are in an aqueous solution, the OH can scramble as the ring opens and closes (see Fig. 6.9).

Note that one of the glucose molecules is reducing. The other lacks the OH on the anomeric center. Overall, these dimers are still reducing sugars and have a reducing end. But the other glucose ring in the molecule is unable to open and close and is not able to undergo reduction reactions.

Other sugars can be linked together in the same fashion as two glucose molecules. Many of these dimeric carbohydrates are quite common in the brewery and used extensively in some recipes. For example, if glucose is condensed with

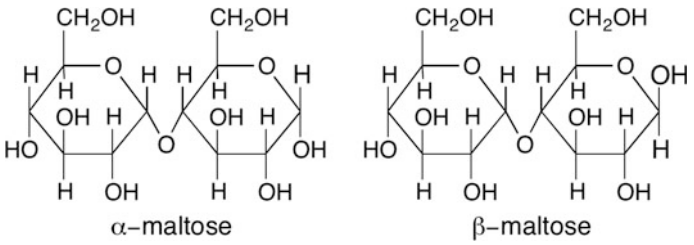


Fig. 6.9 Anomers of maltose. Note that the glucose ring on the right can open and close to give rise to a mixture of α-maltose and β-maltose

galactose by a β-[1 → 4] linkage, the result is the formation of a molecule of lactose. Lactose intolerance results from this linkage. Some people lack the biological machinery to break a β-[1 → 4] linkage. Note that lactose is still a reducing sugar. In another example, glucose can be condensed with fructose (aka fruit sugar) to make a molecule known as sucrose. Sucrose, with an α-[1 → 2] linkage between the two sugars, is not a reducing sugar. We can tell this because it lacks an OH on the anomeric centers (Fig. 6.10).

If we carry the two dimers of glucose farther and create polymers, we end up with the two main forms of glucose that are found in nature. As maltose grows to maltotriose and maltotetrose (adding more glucose molecules), the chain starts to twist around itself into a telephone cord appearance. Every once in a while, a branch comes off of the main chain with an α-[1 → 6] linkage. Thus, the resulting polymer has a very tightly packed form of glucose where multiple chains are packaged in a twisting form that is a great way to store glucose in a system (Fig. 6.11).

If the cellobiose chain is continued, we get a much different form of the glucose polymer. Contrary to the starch molecule, the linkage between the glucose monomers is a β-[1 → 4] connection. This form of the linkage aligns the molecules in a linear zigzag-type arrangement (Fig. 6.12). The chain of glucose molecules just keeps growing and results in a long straight polymer. This polymer, known as cellulose, provides structure to cells in plants.

Another structural polymer of glucose can be found in the cell walls of grains, bacteria, and yeast. Known as β-glucan, the polymer is a slight modification of cellulose. The actual structure of β-glucan varies depending upon the source of the polymer. Yeast cell walls contain β-glucan with β-[1 → 3] linkages between the glucose subunits, and occasionally along the chain there are β-[1 → 6] branches. In cereal grains, the β-glucan contains a mixture of glucose with β-[1 → 3] linkages and β-[1 → 4] linkages (Fig. 6.13). During mashing, the β-glucans in the cereal grains need to be broken down to break the cells walls and free up the materials inside the cells (particularly the starch needs to be released from inside the starchy

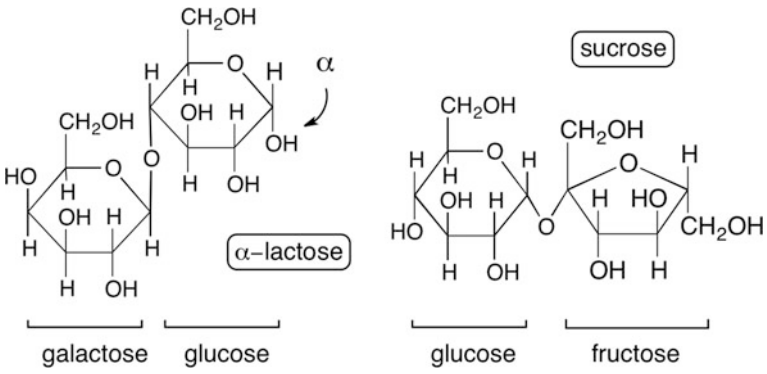


Fig. 6.10 Lactose and sucrose. Note that both alpha and beta forms of lactose exist in aqueous solution, but that sucrose does not have the capability of doing so

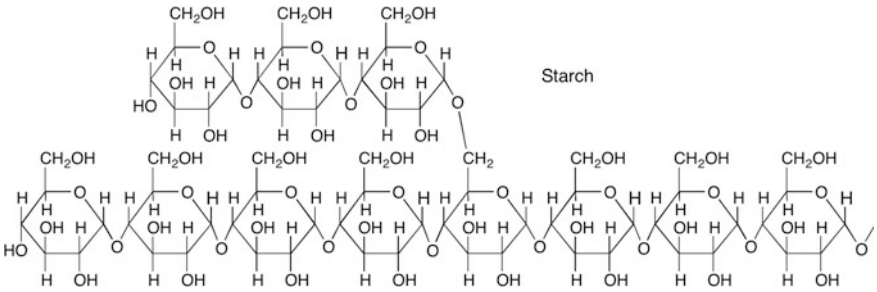


Fig. 6.11 Starch is a polymer of glucose molecules joined with α-[1 → 4] and α-[1 → 6] linkages

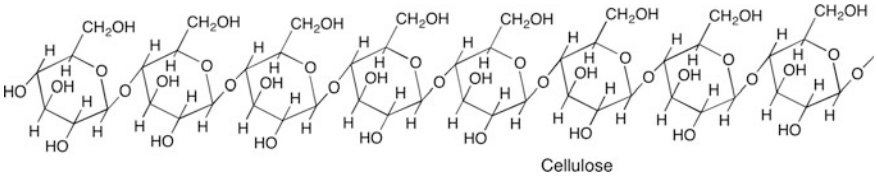


Fig. 6.12 Cellulose is a polymer of glucose molecules joined with a β-[1 → 4] linkage

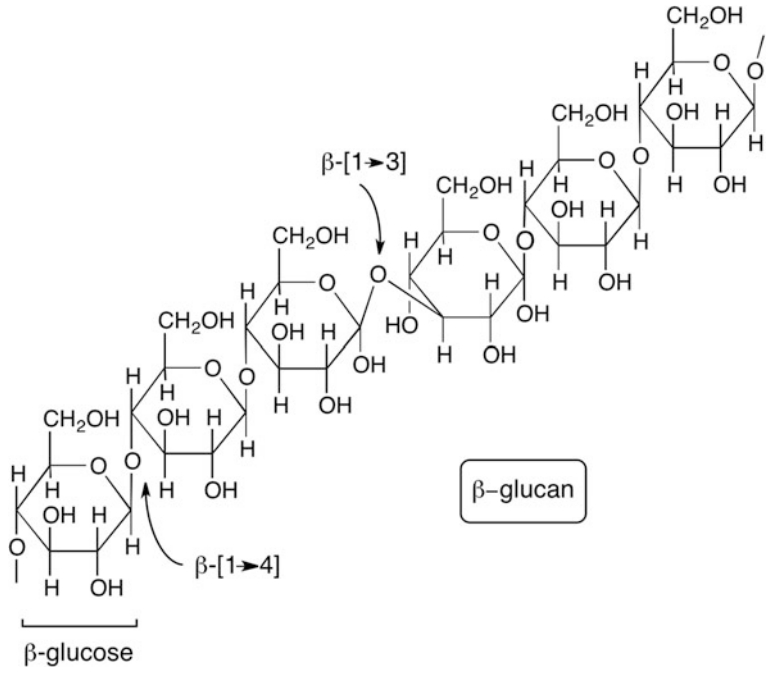


Fig. 6.13 β-glucan is a polymer of glucose. The version shown here is found in cereal grains

endosperm). Because of the differences in the linkages between β-glucan and starch, enzymes have evolved that are specific to breaking each of the bonds in these glucose polymers.

While the words sound similar, β-glucan and gluten are entirely different compounds. Gluten is a polymer of amino acids (i.e., a protein), whereas β-glucan is a polymer of glucose. Gluten does cause some problems with people that have celiac disease. β-glucan, which is not related to gluten at all, does not have this issue.

CHECKPOINT 6.4
Identify the anomeric carbons in lactose and sucrose (see Fig. 6.10).
Galactose and glucose have very similar structures. What is the difference in the structures?
Can you draw L-glucose? Note that it is an enantiomer of D-glucose.

6.4.2 Phytase

Phytase is active in the pH 3–5 range and is active at temperatures ranging from 30 to 52 °C (86–126 °F). This enzyme acts upon molecules that contain phosphate groups. In malt, phytase hydrolyses phytic acid. Phytic acid (see Fig. 6.14) is a molecule that looks very similar to glucose and contains six phosphate groups. This molecule is unusable by the growing barley plant, but when phytase becomes active, the molecule is hydrolyzed to release phosphorus in a usable form for the growing plant. That phosphorus is needed for the construction of DNA, other enzymes, and is involved in the energy production process.

In mashing, phytase removes up to five of the phosphate groups from phytic acid. This results in the primary formation of dihydrogen phosphate ($H_2PO_4^-$), although phosphoric acid (H_3PO_4) and hydrogen phosphate (HPO_4^{2-}) can also be

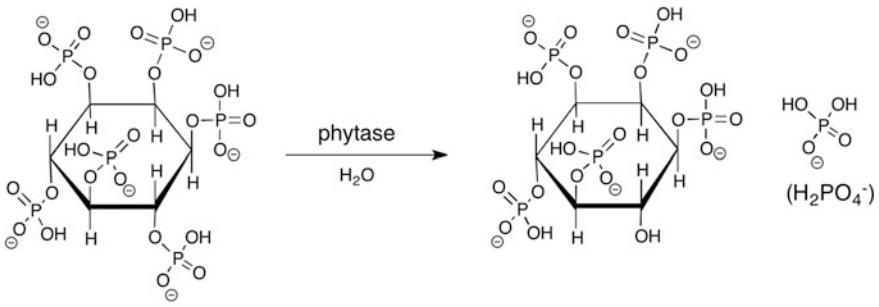


Fig. 6.14 Phytic acid is a source of phosphate. The first release of phosphate is shown here when phytase reacts with phytic acid (here shown as phytate—the penta-anion). Dihydrogen phosphate ($H_2PO_4^-$) is released resulting in a lowering of the pH of the solution

formed based upon the current pH of the water. The result lowers the pH of the solution toward the 4.8–5.2 range. A rest at this temperature, known as an *acid rest*, was historically necessary for brewers that used soft water and malts that tended to be somewhat undermodified. In those situations, the brewer often did not have the ability to control mash pH (or even know about the effect of mash pH). This rest is not necessary for the modern brewery where control of the mash pH is accomplished by adjusting the water chemistry prior to the mash.

With that said, brewers still perform a "dough-in." This occurs when they add the grist and hot liquor so that the final temperature results in a rest near 37 °C (100 °F). As the particles of the grist absorb the hot liquor, they swell in size. The rest here helps the particles get hydrate and limits small ones from falling through a false bottom.

Limit dextrinase is another enzyme that is active in the 32–49 °C (90–120 °F) temperature range and debranches the starch molecule by breaking the β-[1 → 6] linkages, but little of this enzyme survives the kilning process to be of utility. This is not an issue as additional debranching activity occurs later during the saccharification rest.

6.4.3 Proteases and Peptidases

Proteases and peptidases are enzymes breakdown the proteins in the starchy endosperm. It is easy to tell what these enzymes do based on their names. A protease breaks down proteins. A peptidase breaks down peptides (short chains of polymerized amino acids). The result of the action of proteases and peptidases is not only the breakdown of the proteins and peptides, but the release of smaller amino acid chains and even individual amino acids themselves. This helps to free-up the starch so that it can be solubilized. The proteases also break apart any other proteins that exist in the mash and help to thin the resulting beer. Unfortunately, in modern brewing using fully modified malts, an extended protein rests can significantly reduce the mouthfeel of the beer. As we would expect, having some proteins in the beer is important to improve the mouthfeel.

These enzymes are most active in the 4.5–5.3 pH range and a temperature range of 47–54 °C (118–130 °F). A short rest at 50 °C (123 °F) activates these enzymes and lets them do their work. While not needed for a mash containing only malt barley, it can be very helpful for mashes that contain adjuncts such as oats and wheat. Keeping the rest short allows the enzymes to break apart the larger proteins that can lead to haze in the finished beer, but still allowing the mouthfeel of the added adjuncts to show through to the finished product.

6.4.4 Glucanase

There are many different *glucanases* that exist in malt barley. These enzymes act upon the β-glucans found in the malt. As we noted before, the β-glucans are associated with the structural components of the barley seed and are the reason that bread is chewy. Breaking these down helps free up the starch granules for the other enzymes to act upon and gets rid of the "stickiness" of the mash. This is particularly important when adjuncts such as oats or wheat are used in the mash, because these unmalted cereals contain a significant amount of β-glucans.

Specifically, the enzymes work to break both the β-[1 → 3] linkages and the β-[1 → 4] linkages in the cereal β-glucans. The result is that the mash gets a little thinner and less dough-like. In other words, the *viscosity* of the mash decreases. This improves the circulation of the hot liquor during the mash, increases the permeability of the mash when we remove the wort at the next step, and allows the starch to become more accessible during the mash.

The optimum activity of the glucanases in malted barley is in the range of 37–46 °C (100–115 °F). Resting at this temperature for 10–20 min allows those enzymes to do their work. Note that this is the same temperature as the one used for the dough-in. Remember, though, that it is not absolutely necessary to rest at this temperature to force the glucanase to become active in a 100 % malted barley recipe. While it can help thin the mash and break up the β-glucans, the malting process has already done much of the work.

6.4.5 Alpha-Amylase

By far, the most important enzymes in the mash are the amylases. Two important versions of this enzyme exist in malted barley. The first that we will explore is α-amylase. α-Amylase exists in many different organisms; it is found in human saliva and in cereal grains. The structure of the enzyme involves a series of twists like a phone cord arranged into a circle known as a TIM (triosephosphateisomerase) barrel. The result is a central "corridor" along which the starch molecule can be threaded. The enzyme requires calcium to become activated, and once activated it can do its job. Remember that starch is a long polymer of glucose where the linkages are α-[1 → 4].

The enzyme latches onto the long chain of starch and then holds it in place while it performs the reverse of the condensation reaction. In other words, it uses water to break the linkage between two adjacent glucose molecules. α-Amylase cleaves those linkages at random inside the starch molecule. The result is a large number of small glucose polymers. The smallest is glucose itself; maltose is also common, as

is maltotriose, maltotetrose, etc. When the enzyme comes close to a branch in the starch molecules (remember that starch has some α-[1 → 6] linkages), it runs into a problem. The cutting action of the enzyme cannot get close enough to the branch to eliminate it completely. Instead, the enzyme leaves a small twig on the main stem of the starch; the piece left behind is known as a *limit dextrin*.

Some researchers have found that the smallest limit dextrin possible in malted barley involves four glucose molecules bound to each other (Fig. 6.15). Because the structure of amylase differs in other organisms, the limit dextrin structure is also different. Limit dextrins are sweet to the taste and they add a thickness and richness to the flavor of the finished beer. In addition, limit dextrins are classified as unfermentable sugars. That is, limit dextrins cannot be taken up by yeast and converted into energy. Thus, a significant amount of α-amylase activity during the mash results in a heavier mouthfeel and a residual sweetness in the finished product.

α-Amylase activity is greatest in the temperatures from 145–158 °F (63–70 °C) and an optimum pH near 5.7. However, as we noted before, the enzyme is still active at temperatures lower than 145 °F (63 °C). The issue arises when the temperature is too warm and rises above 158 °F (70 °C). This causes the enzyme to denature and become permanently inactivated. As the pH deviates from the optimum 5.6–5.8, the activity of the enzyme also deviates from ideal. Does this mean that the activity drops to zero? No, instead, we find that the activity of the enzyme decreases as we move away from the optimal pH. If the pH of the mash moves

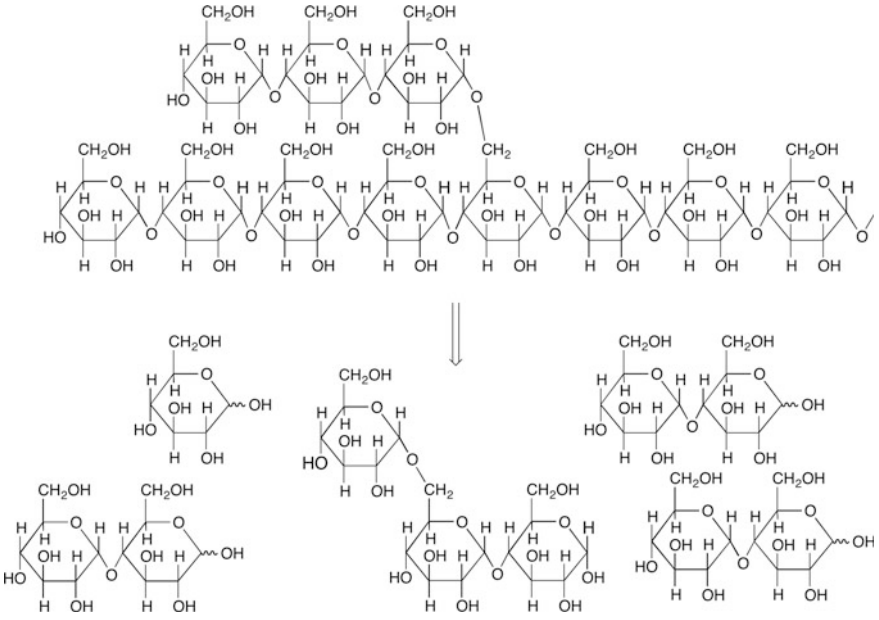


Fig. 6.15 α-amylase activity and the limit dextrin. Note that the major products of the reaction of the enzyme are glucose, maltose, and limit dextrins. The smallest limit dextrin possible from barley malt amylase is shown

significantly far away from the optimal pH, then, yes, a problem would exist. Too far away from pH 5.7 and the enzyme could be permanently denatured or degraded.

CHECKPOINT 6.5
What would occur in a mash that was operated at 60 °C (140 °F) and pH 4.9? What would you expect to happen if the pH of the hot liquor started out at pH 3.0 and 74 °C (165 °F)?

6.4.6 Beta-Amylase

β-Amylase, on the other hand, is a related amylase that differs in structure and function from α-amylase. This enzyme becomes activated during the germination portion of the malting process. Once malted, the β-amylase is ready to go to work on the starch molecule in the endosperm. This enzyme is most active between 131–149 °F (55–65 °C) and in the pH range of 5.4–5.6. It is, as is α-amylase, still active outside of the optimum pH range and at temperatures lower than the optimum range, but the rate of the reaction is much lower.

β-Amylase grabs onto the non-reducing end of the starch molecule and then begins hydrolyzing the α-[1 → 4] linkages between the glucose monomers. The second linkage is the one that is targeted, resulting in the formation of dimers of glucose from the starch molecule. In other words, α-amylase produces significant quantities of maltose from the starch molecule, but only as far as the branching points in the molecule (see Fig. 6.15). In addition, the speed of the conversion of starch into maltose is dependent upon the number of individual strands of starch. The more strands of starch, the faster the conversion of starch to maltose.

So, the best fermentability occurs with a compromise between the temperature and pH for the amylases. Too low and the increased activity of α-amylase becomes active, resulting in a wort that is highly fermentable (with lots of maltose), thinner in mouthfeel, and a beer with high alcohol and very thin feel. Too high of a temperature and the β-amylase predominates, making a less fermentable wort that is thicker in mouthfeel and relatively sweet. The compromise is to find both a pH and temperature that lie in the middle of both amylase activities. Or, the compromise is to ramp the temperature through both temperatures in a stepped temperature mash. For the homebrewer, the best option for the single infusion mash with a cooler as the mash tun is to set the temperature at about 150–152 °F (65–67 °C) and hold it there for an hour.

The production brewery focuses on the saccharification temperatures and slowly ramps the temperature of the mash from 50–78 °C (120–172 °F). This ensures that each enzyme becomes activated at its optimum temperature as the process ensues. In addition, the ramping of the temperature allows the brewer to verify that each of the steps has been reached. If a reproducible temperature increase can be obtained from one mash to the next, the flavor profile can also be reproducible.

6.4.7 Mashout

At the end of the mash, the brewer raises the temperature of the mash to force the enzymes to denature and to denature the majority of the proteins that have become soluble in the liquid. Heat is added to the entire mash. This causes many of the enzymes to even begin degrading and breaking down into their amino acids. The same thing also happens to the other proteins in the mash. They are first denatured. Then, they are degraded and break apart into smaller pieces and even into individual amino acids.

The result of the large biological polymers (proteins and enzymes) breaking down into smaller pieces and individual amino acids is a reduction in the viscosity, or resistance to flow, of the solution. In other words, the liquid gets thinner and the enzymatic activity stops. This allows the liquid to be more easily separated from the grains during the lauter step.

As we will explore in the next chapter, the heated and thinned mash can be separated during the lauter step. The liquid that results is known as sweet wort. The grains that remain, mostly husk material, are known as the *spent grains*, while the sweet wort will later be converted into beer. The destination of the spent grains is often a concern to the brewer. The sugar and most, if not all, of the starch in the grains has been converted into maltose, glucose, and limit dextrins (and a small amount of maltotriose and maltotretrose). Thus, the grains have very little usage left in them as a food source. However, since cattle and other ruminant animals can still digest cellulose-rich materials, the spent grains are unique suitable as roughage (or silage) for those animals. Most brewers donate or sell their spent grains to feed yards, ranchers, or other farmers for this purpose. Alternatively, once dried, the spent grains can be used as a supplement in breads, cakes, and other foods. Some unique uses for spent grains are also being explored (e.g., an abrasive in hand and face soap). Other entrepreneurs might think of additional uses of this material.

CHECKPOINT 6.6
Why is it important to mash-out?
Which enzyme is more likely to make the most maltose?

Chapter Summary

Section 6.1

Mashing is the process by which malt and hot liquor are added together. The process results in the formation of sweet wort containing fermentable and non-fermentable sugars.

Section 6.2

Premash mixers allow the dough-in to start immediately upon mixing of the hot liquor and the grist.
Cereal cookers are required for mashes containing adjuncts that have not been malted.
Decoction and step-infusion mashes allow multiple rests to selectively activate enzymes to accomplish specific tasks during the mash.

Section 6.3

Enzymes are polymers of amino acids that catalyze reactions of molecules.
Enzymes are pH and temperature dependent. If the temperature of an enzyme rises hotter than their optimum temperature, the enzyme will denature and begin to decompose.

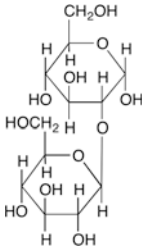
Section 6.4

Starch, cellulose, and β-glucan are polymers of glucose.
The linkages between the glucose monomers indicate the type of compound that exists.
Phytase, protease, glucanase, and the amylases are the major enzymes that are active in the mash. Each performs a very specific function and each is activated at specific temperatures.

Questions to Consider

1. List the reasons why a brewer mashes barley malt.
2. Draw the structure of maltotriose.
3. Identify the fermentable sugars that exist in sweet wort.
4. Why does the brewer cook corn before adding it to the mash? Would this be needed if the corn was malted?
5. Describe a step-infusion mash that allowed the brewer to select the glucanase and β-amylase enzymes only.
6. Draw the temperature profile for a triple-decoction mash.

7. Draw the structure of D-galactose as a six-membered ring and as a five-membered ring.
8. The structure of β-glucan was presented in Fig. 6.13. What would the structure look like if it were part of a yeast cell?
9. Why must a brewer be careful in the amount of time spent during a rest at 50 °C (123 °F)?
10. Determine the amount of mash that should be decocted to raise the temperature of a mash from 50 to 65 °C. Assume the boiling point of water in the Colorado brewery is 95 °C. Would the brewer need to include more or less mash in the decoction if they were at sea level?
11. Compare lactose and sucrose. Which is a reducing sugar and why?
12. What potential problem may arise if the brewer does not perform the "dough-in"?
13. What benefit exists for the use of a premash mixer versus just adding the grist to hot liquor already in the mash tun?
14. Knowing that enzymes denature at high temperatures, why do decoction mashes still make fermentable sugars from starch?
15. Why would a brewer want to do a decoction mash?
16. Many decoction mashes raise the temperature of the withdrawn mash to 72 °C and hold it there for 10 min before boiling it. Why?
17. From Fig. 6.6, what inference can you make about the relative stability of a five-membered ring versus a six-membered ring?
18. Describe the linkage in the disaccharide shown below. Indicate the two monomers that make up the compound and then indicate the linkage.



19. Describe the differences between α- and β-amylases. Which one is responsible for a thinner, more alcoholic beer?
20. Assume a brewer wants to make a very alcoholic beer with a very thick mouthfeel. What should they do during the mash to make this happen?
21. List the major enzymes that are active in the mash and the temperatures at which they are active.
22. Why is rapidly stirring a mash not advised?
23. Consult Table 6.1. Could a mash be performed with ground oats and malted barley (without adding the oats to a cereal cooker before use)?
24. What differences would exist between a mash performed with water that had a pH of 7.0 and one performed using water with a pH of 6.0?

Laboratory Exercises

The Effect of Temperature and PH on Mashing Efficiency

This experiment is designed to illustrate the differences that arise by adjusting the different parameters used in mashing. It further assists with the technical skills used in measurement of the density of wort.

Equipment Needed:

600-mL beaker and 1000-mL beaker per group
2-row malted barley—ground into grist
hydrometer and cylinder
100-mL graduated cylinder
10-mL volumetric pipet and pipet bulb
25-mL beaker
thermometer
hot plate or Bunsen burner setup for heating water with 600-mL beaker
8″ × 8″ baking pan
funnel and filter paper

Experiment:

Each group in the laboratory should take approximately 100 gm of the same barley malt (brewers 2-row will suffice) and place it into the inner beaker of a nested pair of beakers. Water is placed in between the two beakers and will act as an insulator for the mash. The water used in between the two beakers should be as close to the temperature of the mash as possible. The entire apparatus is then placed into a pan containing water at the same temperature as the mash. Then 275–300 mL of pH-adjusted water at the specific temperature is poured onto the crushed grains and allowed to stand for 30 min. Addition of hot water to the pan should be made periodically to maintain the temperature of the mash. The mash is periodically (every 5 min) stirred with a spatula or spoon.

After thirty minutes have passed (use a timer), approximately 75–100 mL, the mash is gravity-filtered into the hydrometer cylinder and then cooled to room temperature either by placing the cylinder in a bath of ice water or by leaving it sit at room temperature on the bench. The density is then determined using the hydrometer. Additionally, the density is measured by pouring $\sim$50 mL into the graduated cylinder and then weighing the liquid. Finally, the density is confirmed by pipetting 10.00 mL of the wort into a clean tared beaker and obtaining its mass. The entire process should be repeated in triplicate and the average density reported for the sample.

Different groups of students should use different temperatures for their mashes. The table below indicates a set of temperatures that can be used to provide information about the activity of the saccharification enzymes. In addition, the pH of each

Table 6.2 Temperatures and pHs to explore in a class of 20

Trial	Temperature (°F)	pH
1	100	5.4
2	125	5.4
3	150	5.4
4	175	5.4
5	125	6.0
6	125	4.0
7	150	6.0
8	150	4.0
9	175	6.0
10	175	4.0

Additional students can duplicate trials 1–4

group's water used in the mash (no adjustment needed to the water between the nested beakers or in the pan is needed) is set to a specific pH. The pH can be set by using phosphate buffers at the specific pH or by simply adjusting the pH of tap water using dilute phosphoric acid solutions (Table 6.2).

After the entire class has determined the density of their specific trial, the results are compared and then discussed. If multiple groups perform the same trial, those results are averaged before the discussion.