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Enzymes in Brewing

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The subject of enzymes in brewing is often constrained to the mash vessel. It is the objective of this article to expand this understanding to include the malting process and how it relates to beer, as well as to bring in the commercially available enzymes for consideration. By appreciating all of these areas, it is easier to understand the magnitude of this complexity. To consider one area without considering all others leaves gaps in understanding and thus opportunities for issues to arise. Thus, this review covers enzymes from malting through to commercial enzymes. Working in the field of commercial enzymes has taught me a great deal about the world of naturally occurring enzymes. Appreciating the full scope of enzyme activity is important to understanding how to influence process change. The approach of this article is to discuss how the enzymes relate to specific aspects of the process. It is up to the reader to integrate this complexity into the other related processes in the brewery.

In this review, we look at enzymes from malt to commercial sources. The process of malting converts the hard grains of choice to softened grains with available extract and the needed chemistry to convert this extract into a useful basis for yeast to make beer. The process of selecting grain, malting techniques, and mashing processes utilizes a full understanding of the enzymes needed in your process, especially if you run on the edges of the process capabilities. We will begin with a discussion of enzymes and their activation in malting. We should note that the malting process utilizes these naturally occurring enzymes within the grain to free up the extract for our use. Within the context to follow, I will be referring to the process of malting barley. It should be noted that many other grains used in brewing can also be malted in a similar manner, thus making extract more available to the brewing process. In addition to freeing up the extract, the malting process gives the brewer the enzyme spectrum required to make the product fermentable, thus the basis of beer. We will then move through the enzymatic processes in mashing. Because this is a highly integrated process of time and temperature relationships, the effects of one change can have profound impacts on other aspects of your process. In reviewing the enzymes in this process, we can begin to appreciate the range of these impacts. Lastly, with today's focus on being different or looking at the very edges of what a process might be capable of, we review the world of commercial enzymes available in brewing. Other factors besides stretching the limits of a process may influence your interest in the use of commercial enzymes. Problems in malting caused by grain issues such as preharvest sprout, or variations in growing conditions affecting protein or carbohydrate levels, can be effectively dealt with by using some of these products. The intent of this discussion is not to suggest that you use these products but to explain how they can be helpful under specific circumstances. Knowing the whys and the hows can add to your brewing toolbox.

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Malting

Malting, the process of converting barley into malt, is as old as the history of beer. Malting and brewing are based upon the presence of carbohydrates and plant protein in the barley kernels, the ability of malting to create the natural carbohydrate-reducing and protein-reducing enzymes, and the production of both simple sugars from the plant starch and an acceptable protein spectrum from the plant proteins by utilizing these enzymes. Malt is a product of the plant germination process within a set of controllable parameters required to achieve consistent results for the brewer.

Malting Process

The malting process consists of three phases: steeping, germination, and kilning. After the plant growth process is activated through steeping and germination, the green malt is kilned (dried and roasted) to stop the growth of the barley seedlings and to develop malt flavor and color.

Carbohydrates

Common carbohydrates that we are familiar with are sugars, starches, and cellulose. The basic building block of all carbohydrates is a simple sugar, glucose. All carbohydrate chains are chains of glucose bonded together. Starches are composed of the fermentable α-glucose, and cellulose is composed of the nonfermentable β-glucose. Unless specifically referenced beyond this point, all glucose is to be considered α-glucose.

Plant starch is the stored energy of the plant that is broken down by plant enzymes when the plant requires energy. In simple terms, the starch found in the barley kernel is of two types: amylose, containing linear chains of glucose molecules, and amylopectin, which is a multibranched structure of glucose chains. Amylose and amylopectin differ in how the glucose units are linked together. Both have 1-4 glucosidic bonds within their linear structure holding the glucose molecules together, but amylopectin also has 1-6 glucosidic bonds at branch points, creating its complex structure. This difference is important to the understanding of how the breakdown of the glucose chains leads to a difference in extract and fermentability.

Proteins

Proteins are the basic raw materials in tissue and cell building essential for life. They are part of the matrix that holds the cell together. Additionally, they supply the functional molecules for sustaining life within the kernel. Like carbohydrates, proteins are complex chains of the simplest protein compounds, amino acids. Unlike carbohydrates, which all are built from a single compound (glucose), proteins are built from 25 different amino acids. Proteins are essential to brewing to pro-

vide the amino acids for yeast nutrition, as well as to provide flavor, mouthfeel, foam, and color. Larger proteins can also lead to turbidity and sediments.

Enzymes

Enzymes are polymers of amino acids and for all intents are also proteins. They are proteins with a function. They are catalysts that regulate the speed of chemical reactions involved within a living organism's metabolism, without permanently changing the reactions. The enzymes of our particular interest are the ones responsible for digesting the starch and protein chains described earlier. The prominent malting and brewing enzymes are, but are not limited to, amylases (carbohydrate enzymes), proteases (protein enzymes), peptidases (which break down protein pieces into amino acids), and β-glucanases and xylanases (cellulose enzymes). There are several additional enzyme classes that have little to do with the processes described here and will not be considered in this part of the discussion. We will discuss some of these enzymes later as we review mashing and commercial enzymes. In the review of malting we will not cover the actual process of malting. Our focus will remain on the enzymatic processes. Understand that malting is a complex process of air flows, temperatures, bed depth, and moisture control designed to facilitate the controlled growth of a barley plant. Our focus here is to look at the steps in malting to see how the enzymes become available for the brewer.

Steeping

As stated earlier, the malting process consists of three phases. The first phase, steeping, is a series of full immersions of large batches of barley in constantly aerated temperature-controlled water, spaced by drain cycles, again with heavy airflow to prevent heat and CO_2 buildup. The purpose of this process is to increase the internal moisture of the barley kernel to above 40% while not drowning the early growing plant or damaging it by heat or CO_2. This increased moisture signals the internal mechanisms in the plant to begin growth, and the moisture provides a medium for the transfer of the enzymes inside the kernel to begin self-digestion. Thus, the growing begins. This process can take upward of 48 h, depending upon the quality and type of the barley, the temperatures of the various steep cycles, and the temperature of the grain and the air applied. Additionally, barley fresh from harvest and sometimes barley that has been stored a long time can exhibit a delayed response to this hydration. This delayed response is known as dormancy (a natural process set up by the organism as a protective response to keep the seed from trying to germinate under harsh conditions). Care in hydration of the kernels needs to be exercised when dealing with this phenomenon. The biology of the kernel sets the process in motion. As the plant begins to sprout it requires energy to grow. The energy comes from the starch stored in the seed. To get to this starch, the plant creates a series of enzymes designed to digest the supporting materials holding the starch, and later the starch as well.

Germination

The second phase of malting is germination. The steeped barley is transferred into large growing beds and leveled to provide equal exposure to the environmental conditions applied by the maltster for control. The germination process is a 4–5 day process that facilities the growth of the barley seedling. Large amounts of moisture-saturated and temperature-controlled air are cycled through the beds to facilitate the removal of heat and CO_2 generated by the now respiring plant. Excess of either will damage the growth process and create a whole set of other issues such as premature yeast flocculation and poor or variable modification, along with the potential for kernel death. While the barley is in the beds, turning machines are sent regularly through the growing barley to keep the rootlets from matting as well as to apply water as if to water a garden. What the maltster is attempting to do is to control the growth of the emerging plant while allowing the development of the enzymes needed for digestion. This digestion is focused on making the materials available from this stored matrix for plant growth. A sequence of enzymes is released by the germinating plant, principally proteases to begin with and some β-glucanases and xylanases, all focused on digesting the matrix around the starch granules stored in the kernel. Eventually, α- and β-amylases are released to begin the digestion of the starch to provide energy for the growing plant. The key to the maltster's objective is to control and limit the amount of starch digestion while maximizing the amount of digestion of the matrix of protein, β-glucan, and xylan holding the starch granules together. This is referred to as malt modification.

Kilning

After the controlled growth, the green malt is transferred to a kiln, where heated dry air is applied to dry the grain in such a manner as to stop growth while preserving as much of the enzyme activity as possible. In the later stages of drying, the heat is increased and the flavor and color development is enhanced. Various methods and times of heat application are used to facilitate broad ranges of color and flavor used by the brewer. How the drying cycle is approached will have a great deal to do with what level of enzyme activity remains in the grain. Obviously, the more heat applied, the less remaining enzyme activity. What is less obvious is that a higher temperature in the presence of moisture has a greater effect in destroying the enzymes. The challenge is to balance flavor versus functionality.

Self-Digestion

During the growth of the barley kernel, which is occurring in the late stages of steeping and throughout germination, the objective of the growing plant is to self-digest its kernel to provide energy and nutrients for growing. This self-digestion occurs through the growing plant's creation of a series of enzymes designed to break down the kernel's starches and proteins. The maltster's objective is to control and harvest this process. In simple terms, the amylase activity for starch breakdown is minimized to prevent fermentable extract loss for the brewer, while the β-glucanase and xylanase (cellulose breakdown) and protease/peptidase (protein breakdown) activity is maximized to soften the kernel and expose the starch for extraction in mashing. Obviously, a great deal of expertise and control goes into this process.

Analytical Outcome

Coming out of the malting process, we have now modified the barley to have a level of carbohydrate-digesting enzymes, expressed as α-amylase content and diastatic power, within the maltster's analytical profile of their finished product. Additionally, we will see an increase in soluble protein and, more importantly, an increase in the soluble-to-total protein ratio as a direct expression of the barley modification. Lastly, we will see a reduction in the β-glucan content and the wort viscosity as an expression of cellulose digestion. Obviously, if your independent focus was only to consider extract, you would be

looking to maximize each of these changes. But as we all know, what is important is balance. This balance provides for the needed economies of our processes while facilitating control and consistency in creating the variety of products we enjoy. There are some informative articles previously published in the *TQ* that reflect the importance of balance in all of these processes (2–4). Additionally, they give an outstanding picture of the outcomes of each of these processes.

Mashing

Process and Objectives

In this part of the discussion, we will consider the mashing profile of a single-malt mash. Obviously, there are many different methods to mash and additional materials that can be added. Each in itself can add complexity to our understanding. However, if you understand the basics, much of this is transferrable to other methods and materials, as long as you understand the specific impacts of each variation. For instance, decoction brewing is a wonderful process to build flavor. But one needs to note that each pump-over and boil results in a loss of enzymatic power. Additionally, during pump-back, if you do not use extreme care you also will lose enzymatic power owing to thermal degradation within the proximity of the hot liquid until blended. It is not that these processes cannot be used; one just needs to understand the constraints. Only 15–25% of barley malt is soluble in water. Mashing is the process during which a much greater fraction of the malt is made soluble. During the process of mashing, a predetermined fraction (based upon the product definition by the brewer) of the solubilized compounds is converted from starch to yeast-fermentable sugars. The end product of mashing is wort. Wort contains fermentable and nonfermentable carbohydrates, proteins, amino acids, and other organic and inorganic compounds that are extracted from the malt. The primary goal of mashing is to produce as much high-quality extract as possible from the grain, with some desired fraction of this extract being converted to fermentable sugars, which will eventually lead to making alcohol in the presence of yeast during fermentation.

Amylose and Amylopectin

Earlier we discussed starches as a mix of amylose and amylopectin. Barley malt contains about 60% starch. Approximately 75–80% of this starch is amylopectin, the complex highly branched (1-6 glucosidic bonds) polysaccharide that can contain over 100,000 glucose monomers, with 20–25 glucose unit strands (1-4 glucosidic bonds) between the branch points. The remaining 20–25% of the malt starch is amylose, linear strands (1-4 glucosidic bonds) of 1,000 or more glucose monomers in sequence.

Fermentable Sugars of Starch Digestion

When we discuss the digestion of these starches we break the resulting molecules into two fractions: those that are fermentable by yeast and those that are considered nonfermentable. The fermentable sugars created from barley malt via enzyme digestion are glucose (single monomer), maltose (dimer of glucose joined by 1-4 glucosidic bonds), and maltotriose (trisaccharide of glucose that is joined by 1-4 glucosidic bonds), and that is it! One should note that not all yeast will ferment maltotriose, or at least not all of it. So when determining your fermentability from a sugar profile, your yeast's capabilities to deal with maltotriose need to be considered. A good basis to start from is to consider not more than 50% of the maltotriose as fermentable. We should also note that there are other sugars that yeast will ferment, namely, fructose and sucrose. These sugars do not originate in malt; they may enter your process through the addition of other fermentable materials such as honey, fruits, and table sugar.

Free Amino Nitrogen

Earlier, we introduced a discussion on digested protein and its importance to yeast growth. In barley the protein content can vary from 7% to about 15% by weight. About 30% of this protein will pass through into the finished beer, primarily as breakdown products of the initial complex protein structures inside the barley kernel. Protein breakdown products are amino acids (single monomers of these nitrogenous compounds) and peptides (short chains of amino acids). As previously noted, proteins and high-molecular-weight peptides are important to beer foam and body, but they can also result in haze. Smaller peptides are basically neutral with no discernible effect. However, amino acids are vital yeast nutrients often referred to as amino nitrogen or free amino nitrogen (FAN). The amount needed is a direct reflection upon the amount of yeast growth that occurs in your process. When digesting proteins to amino acids, we need to consider the classification of these amino acids. The 25 different amino acids can be broken into four categories (1,8). The first category is absolutely essential for yeast growth, the second is important, the third is less important and used only if needed, and the fourth is not used in anaerobic processes. Therefore, in simple terms, whatever amount you start with for FAN, you need to ensure that you will not use it all. Expect at least 25–30% and preferably closer to 50% to pass through as unused and unwanted FAN. A good starting number would be 1 ppm of wort FAN needed for every 1 million yeast cells you grow. Therefore, a safe position would be a starting FAN of 200+ ppm if you were going to grow 100 million cells, thus leaving you with around 100 ppm. As your yeast progresses through the second and into the third categories of amino acids, you will begin to see other issues develop in your flavor profiles. These issues are typically associated with excessive sulfur products being generated as well as a propensity for sluggish or incomplete fermentations.

Enzyme Overview

Moving on to enzymes, we also previously noted that they are high-molecular-weight complex proteins that act as catalysts for organic reactions. Typically, a catalyst enables a reaction to occur or considerably accelerates the rate at which it occurs. Enzymes are extremely specific, catalyzing only one chemical reaction. There is a whole science around how enzymes work, beginning with a naming convention. In simple terms, the substrate affected by the enzyme becomes the basis of the enzyme's name, with an "ase" attached to the end. Thus, proteases affect protein, β-glucanases affect β-glucans, and so on. The science of enzymes theorizes a lock-and-key concept of attachment and reaction, feedback and regulation, environmental effects, and so on. For our discussion, we are really only interested in the effects of time, temperature, and pH. Enzymes tend to have high specificity to the effects of temperature and pH. Additionally, as the limits of these are reached, time becomes the major driving factor in the remaining activity and net performance of the enzyme. For a greater understanding, please see P. R. Mathewson's book *Enzymes* (6).

pH

As complex protein structures, enzymes possess clearly defined polarized (charged) areas. This polarization determines much of their shape and can be a major part of how they function. The ion characteristics of the solution they reside in will have a major effect upon their polarization, therefore affecting their ability to hold a shape and function. In basic terms, the pH of a solution expresses the balance of negative and positive ions. In looking at mashing, we normally look at a pH range of roughly 5.0–5.7. For the most part, the natural enzymes we are concerned about in mashing function well within this range. So for the sake of this discussion, we no longer need to be concerned. There are some specialty mashing processes that take us outside this normal pH range, resulting in opportunities for greater discussion.

Time and Temperature

We will consider in greater detail what we are trying to achieve in the mashing process. In mashing our objective is to influence the enzymes created in malting to digest the barley malt extract in such a manner as to make the wort for the beer type we are trying to create. To accomplish this objective we use the variables of temperature and time to establish that influence (again, as required for the beer we are making). In simple terms, all enzymes have a temperature of greatest activity. Unfortunately, this high rate of activity normally occurs close to the temperature at which the enzyme becomes deactivated. Therefore, time also plays a role. The closer we get to the optimum activity temperature for the enzyme, the shorter the life span is for the enzyme. Thus, balance is needed, as well as an understanding of which enzymes are critical at each stage in mashing.

Seven Critical Enzymes

With this background we can now move into the mashing process. As stated, this process is a sequence of times and temperatures designed to influence the enzymes needed for the beer being made. There are many enzymes that are active within a mashing process. Many of these enzymes act on substrates that are either in such a large quantity that their very level defines a process, or the substrates are so small they can be considered *de minimis*. For the sake of this discussion we will consider only the seven enzymes that are really important to control for our processes.

The First Three Enzymes

Normally, mashing begins at a certain temperature for one of two reasons: it is designed to fit our equipment, or it is believed that there is some benefit. In any event, it is important to fully wet the grains and begin the process of freeing up the enzymes into solution. Three of the seven enzymes concern us at mash in. These three enzymes have optimal activity temperatures around where most brewers begin the mashing process, 100–120°F. These enzymes are β-glucanase (optimal 113–122°F, deactivated at 140°F), ferulic acid esterase (optimal 100–113°F, deactivated at 149°F), and proteases (optimal 113–130°F, deactivated at 158–167°F). As you can see, some optimal activities overlap in temperature, which results in multiple activities occurring at the same time at specific temperatures.

Protein Rest Benefits

If the maltster began with great barley and has done their job correctly, we have well-modified malt and therefore little concern about β-glucan and FAN content. However, in the real world we know this is not always the case. It should be noted that after kilning much of the β-glucanase and protease capabilities in the malt have been eliminated. Therefore, attempting to further reduce β-glucans or increase FAN by using extended protein rest holds around 120°F will have only a small effect (perhaps about a 10% shift). What is critical to note is the ferulic acid esterase activity. Extended holds can result in an increase of upward of 100% in ferulic acid.

Ferulic Acid

Ferulic acid by itself is not of great concern (Fig. 1). However, if your wort possesses high levels of ferulic acid and your process allows for long hot temperature holds after the kettle boil, you may experience a flavor impact on your beers. As ferulic acid sits at a high temperature, it will autoconvert to 4-vinyl guaiacol (4VG). 4VG is highly volatile and possesses a strong clove aroma and flavor. For some beers this flavor is considered normal, but for many beers it is considered a defect. Therefore, if you are short on FAN or long on β-glucans, there are commercially available solutions to address these issues. Otherwise, attention to the process becomes vital to maintaining a 4VG-free product.

Conversion Temperatures

Moving further along in the mashing process, we normally see the use of one or two temperature holds to influence the optimal activity of the starch-converting enzymes. This step is typically known as the conversion hold. Our objective is to create a blend of fermentable and nonfermentable sugars for the beer type we are making. The next four of the seven enzyme groups now become of interest. We can break these down into two basic categories. The first three are starch-converting enzymes: limit dextrinase (optimal 131–140°F, deactivated at 145°F), β-amylase (optimal 140–149°F, deactivated at 158°F), and α-amylase (optimal 162–167°F, deactivated at 176°F). The last enzyme of interest at these temperatures is β-glucan solubilase (optimal 140–158°F, deactivated 163°F), which by reference to its name solubilizes β-glucans.

β-Amylase

β-Amylase is the first starch-digesting enzyme we will consider. Typically, the ratio of fermentable to nonfermentable sugars in a mash is set by the conversion temperature. This temperature should be chosen to express the blend of the two

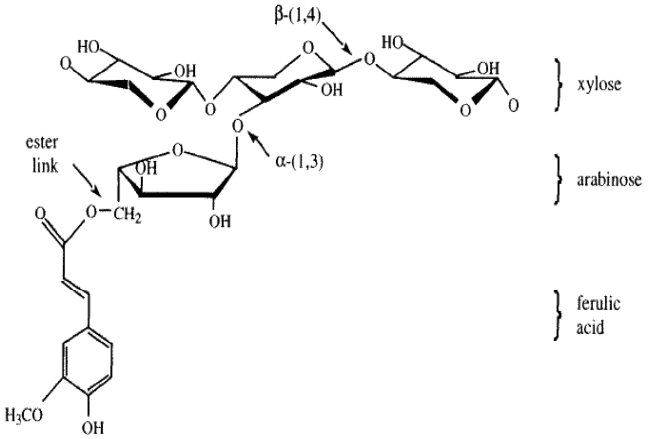

Figure 1. Arabinoxylan and ferulic acid. Image courtesy of DuPont.

principal starch enzyme activities: α- and β-amylase. The β-amylase digests strands of starch and dextrins, breaking them down to maltose molecules and undigestible fragments. As it works, it can only break linear 1-4 glucosidic bonds and therefore cannot digest amylopectin owing to the branch points. In addition, it can only address the starch chain from one end, the nonreducing end. Therefore, its activity is one molecule at a time, working along the chain until it reaches a branch point, where it must stop.

α-Amylase

For β-amylase to be effective, more nonreducing ends must be made available for its attack. This is where α-amylase comes into play. α-Amylase also only breaks 1-4 glucosidic bonds, but it can attack the starch molecule at any point. This random attack results in a large array of smaller dextrins and a small amount of fermentable sugars.

Sense of Balance

It would be great if it were that easy. But note, the optimal activity temperature for the β-amylase is much lower than for the α-amylase. It is therefore possible to deactivate the enzyme that makes fermentable sugar before the α-amylase can break down the starch for β-amylase attack by freeing up nonreducing ends for the enzyme to attach to. Thus, we need some balance. Keep in mind that most enzymes are active at some level up to their deactivation temperature. They are just not as active as they are at their optimal temperature. So at the optimal temperature for β-amylase, 145°F, α-amylase is digesting starch, just not at the same high rate it would be if the temperature was 165°F, roughly seven degrees higher than the deactivation temperature of β-amylase. Shifting the rate of temperature increase and the actual hold time (if used) can influence the ratio of activity of the enzymes and thus affect the end ratio of fermentable to nonfermentable sugars. Highly modified North American malts possess extremely high diastatic power (starch-reducing capacity). In all-malt mashes, fermentability can be established by the rate of temperature increase, often negating the need for an actual conversion temperature hold.

Time

Given this concept of balance, we must now introduce the variable of time into the discussion. The β-amylase works by chewing up a linear strand of starch or dextrin, one maltose molecule at a time. On a relative basis, it is a fairly slow process. The α-amylase cares little about where it attacks the starch, and therefore it is considered relatively fast. If brewers are looking for more fermentability (higher alcohol, thinner, and faster beer), they will need to place the conversion temperature closer to the optimal activity of the β-amylase. Additionally, they will need to consider the relative rate of reaction, and the hold time for the conversion will need to be longer, again on a relative basis. Conversely, if they are looking for less fermentability (lower alcohol level with greater body and sweetness), they would choose a higher temperature to favor the α-amylase activity, while carefully considering the deactivation temperature of the β-amylase and noting that the reaction will occur quickly.

Words of Caution

As you look at the opportunity before you, I offer a couple words of caution and advice. When dealing with mash temperatures close to or below 147°F, one needs to consider the microcharacteristics of the process. As you drop near to 145°F you are now allowing for the incubation of a large amount of naturally occurring microflora that come with the grain. This is nothing to worry about, unless you hold for extended periods of time, as with long holds to create highly fermentable beers. Holds that exceed 3–4 hours can be problematic owing to the microflora contributing vegetable, especially radish-type, flavor notes to your beers, so be cautious. In addition, the longer you hold, the more damage your filter bed will take. Therefore, with longer conversion holds consider coarser grinds and slow agitation speeds.

Our Last Two Critical Enzymes

To finish our discussion, we need to look at the last two of the seven enzymes we discussed: limit dextrinase and β-glucan solubilase. Keeping with the starch discussion, we will look at the limit dextrinase first. This enzyme possesses the ability to break the 1-6 glucosidic bonds in the branch points of amylopectin. As with most enzymes of its type, it is extremely temperature sensitive and is deactivated rapidly at or above its deactivation temperature of 145°F. Additionally, there is little of the enzyme to begin with in barley and much less, after kilning, in malt. However, it does exist and does have an impact, albeit an insignificant one as relates to the amylase activity. I mention it here to help stimulate thoughts of "what if" as we look forward to the section on commercial enzymes. During that discussion we will refer to limit dextrinase as pullulanase, as these enzymes are typically known within this industry. As you ponder scenarios, consider that there are other tools available to help you achieve what you are trying to accomplish.

β-Glucan Opportunities

Lastly, we look at β-glucan solubilase, a problematic enzyme when considering malt that may be borderline in modification (slightly high in β-glucans). Additionally, longer holds for conversion or delays in your brewhouse process, holding up your lautering, can also play into this enzyme. Earlier we mentioned the optimal activity temperature of this enzyme at 140–158°F. Interestingly enough, this temperature range centers closely on the range most brewers consider for conversions. Most brewers will mash off to lauter/strain at temperatures at or greater than 163°F, close to the β-glucan solubilase deactivation temperature. But often if brewers are stretching the limits of the β-amylase activity, some will attempt to mash off in the 150s, leading to extended time for the β-glucan solubilase to work as well. From its name, this enzyme solubilizes β-glucan, freeing it from the husk material, not only allowing more into solution but also further degrading the integrity of the filter bed. It is a quandary on how to deal with this enzyme, except to offer two solutions if you venture down the path of long holds. The first deals with extremely well-modified malt. Keep in mind this is not standard material available to the public: one must request and probably pay for it. To do this, maltsters must play with the maximum ranges of processes that can stress the grain and create or flirt with premature yeast flocculation potentials in the malt. So if you take this path, understand that there are no solutions to premature yeast flocculation yet, other than to avoid it and apply a general-use commercial β-glucanase in your process (see more later).

Expanding Your Knowledge

For a greater understanding of enzymes in malting and brewing, read the two articles reproduced in this issue of the *TQ*: "Development of Enzymes During Malting and their Func-

tion in Mashing" by Neville Prentice and "Biochemistry of Malting" by B. R. Sebree (7,9). I have utilized these two references for much of my career and find them to be very informative. For additional considerations, also see an excellent *TQ* article by L. Narziss (5) and a *JIB* article by Vriesekoop et al. (10).

Commercial Sources

We have discussed the basics of creating the enzymes in malt for the brewer, and then mashing with those enzymes, targeting specific objectives within those processes. As we progress through this final section we will begin with many of the same enzymes discussed earlier, but these are now coming from nonmalt sources. Typically, these are derived in fermentations similar to brewing culture operations, but the organisms producing the enzymes are either bacteria or fungi (that are not brewer's yeast). As I progress through each of these enzyme groups I will not reflect on specific benefits of using one company's product versus another. It should be noted that often they are extremely similar, but sometimes they are quite different, primarily because of the various host organisms chosen to make the enzymes. In nearly all cases the enzymes marketed by these companies possess smaller side activities of additional enzymes. These activities can be beneficial or not. Because this is a natural process and the organism is influenced to produce the specific enzyme of interest by influencing its environment, the influence applied cannot totally prevent the organism from making additional enzymes. This is why you may see side activities. Some companies will claim them, others will not. In most cases the activity levels are not determined or regulated within their processes. When looking at these products it is wise to ask about side activities as well as conducting bench-top mashes for evaluation of outcome. Also, within this discussion I will not reference dosing rates because these too will vary from product to product. For the purpose of keeping this discussion to a reasonable length I will not consider the range of products to use if you are looking at very high additions of or are brewing with specialized grains such as sorghum or greater than 50% unmalted grains. We will, however, touch upon the use of barley, rye, and wheat, because these additions can pose significant challenges to any brewer. The choices you make when looking for help via the use of commercial enzymes can be greatly influenced by knowledge about the specifics of these grains.

Commercial Enzyme Categories

Most commercial enzyme companies break their products into several basic areas. The first two major groups deal with attenuation control and with optimization of mashing and beer filtration. The next major group often is associated with cost-effective cereal cooking. However, many of these enzymes can also be used in improving your attenuation control. Sometimes enzyme classifications may roll over from one area to the next. The next groups we will discuss get more specific. These groups fall into categories that are often called cost-effective adjunct and malt opportunities, optimal or improved fermentation control, specialty grains, and finally one very specific classification that can address improved haze control. In almost all circumstances the enzymes are added in the main mash or cooker mash unless otherwise indicated. In most cases it is important to deactivate the enzymes in your kettle boil, as is done with natural malt enzymes, thus stopping any changes driven by their activity in your products.

Attenuation Control

The largest family of enzymes available for commercial use is categorized typically under attenuation control. You will also see that some of these will be categorized elsewhere as well. These too can be broken into families: amylases, glucoamylases and amyloglucosidases, and limit dextrinases (pullulanases) (Fig. 2).

Attenuation Control: Maltose-Producing Amylase

Earlier, we looked at the natural occurrence of α- and β-amylases in malt. These two enzymes are available in several forms commercially. As we walk through these, consider your processes, because there may be several reasons to consider the use of these enzymes. These enzymes can increase the rate of reaction, increase the yield, and help you attain your end alcohol targets, especially if you are using either poorly modified malts or cereal grains lacking in enzyme power. To begin, β-amylases are hard to come by. Typically, this enzyme is only commercially available as a malt extract. It is not widely used within the brewing industry because of its cost and the availability of other products. There is currently only one other alternative to β-amylase, and this is a widely used product: fungal α-amylase. Although this product is very temperature sensitive, it works similarly to malt β-amylase in producing maltose as it digests starch. There has been industry discussion around this area, and I believe some progress has been made. This is a changing industry. Staying aware of changes is recommended if you have a need or interest. If you need to tune a conversion, these products can help maintain a maltose-based wort, thus avoiding glucose-suppressing issues with high real degree of fermentation (RDF) creating products. If you are unaware of glucose suppression, it is a serious issue when looking to work with high-RDF products. I will review this in some detail later in this discussion.

Attenuation Control: α-Amylases

Moving on into the α-amylases, there is a vast range of these. Some that are available will have similar sensitivities to temperature and pH as the malt α-amylase, and you can look at these as enhancements. However, there are other amylases for mid and high to very high temperatures that were developed primarily for the syrup industry. These products also come as blends or individual products. For brewing, the use of

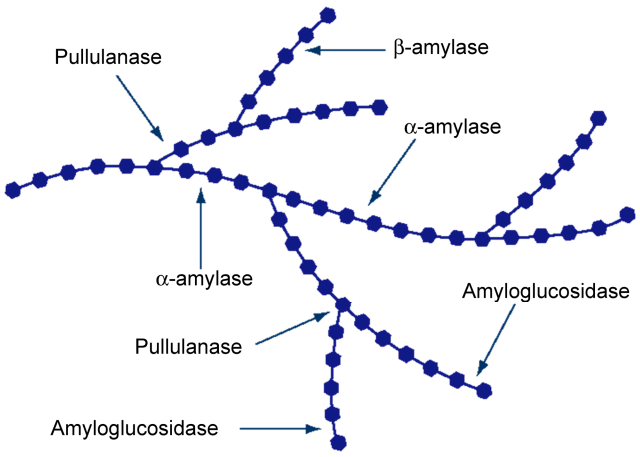

Figure 2. Starch-reducing enzymes, showing what portions of the molecule they work on (⬢ = glucose). Image courtesy of DuPont.

blends makes good sense in that you can reduce viscosity earlier at a lower temperature and then at the high temperature the enzyme will provide extract and so on. This sort of discussion falls closer to the use of adjuncts and the liquifaction and gelatinization of the starches in the cooking process. However, for attenuation control, and more closely related to malt processes, these amylases can be added as a supplement to processes that need additional amylase power to get a reasonable conversion within a reasonable time.

Attenuation Control: Glucoamylases

The most widely used products for attenuation control within brewing are in the family of enzymes known as glucoamylases. You may on occasion hear these referred to as amyloglucosidases, but for the purpose of this discussion you can consider these to be the same. Glucoamylases attack starch molecules from their nonreducing end, clipping off single glucose molecules one at a time. They are capable, for this discussion, of only breaking the 1-4 glucosidic bonds and therefore cannot proceed past the 1-6 branch points in amylopectin. When used with well-modified malt (an ample supply of α-amylase to break up the starch molecules, especially past the 1-6 branch points) these enzymes can easily achieve a very high degree of fermentability. Under nonspecial circumstances, RDFs over 80% are not difficult to attain. Values approaching 85% are fairly easy with some special considerations, and values as high as 87% have been achieved in all-malt mashes. I mentioned glucose suppression (or repression, as referred to by some) earlier, and typically such issues can come into play with the use of glucoamylases. With the use of glucoamylases our objective is to convert most of the starch and dextrins to glucose. If you do this and get most, perhaps 70%, of it converted over to glucose, you will normally not see an issue. The glucose suppression issue comes with using small amounts of glucoamylase as an enhancement to an RDF that is naturally attained. A naturally based RDF will produce mainly maltose, normally greater than 60% as such. If you are using well-modified malts, it is not abnormal to naturally achieve RDFs just under 80%. Wanting to get just a little more is where the problem lies. Once you convert some of the sugars to glucose, you begin to enter a no man's land of sugar ratios for yeast fermentation. Most yeast will start to have issues when you approach 25–30% glucose. The problem comes when the yeast finish the glucose and then must either continue to ferment or shut down. If the fermentation shuts down, it can get interesting. You can have unfinished products, elevated diacetyl, and so on. When considering all of these processes and products, knowledge and understanding are paramount to your success.

Attenuation Control: Limit Dextrinases (Pullulanases)

One of the more interesting groups of enzymes available is limit dextrinases (pullulanases). For the sake of consistency, I will refer to them here as pullulanases. These enzymes possess the ability to break the 1-6 branch points in amylopectin. One might think this enzyme would have a major impact on RDF. However, in reality, because of the normal activity of the α-amylases, there is not. We can, however, use these either alone or in conjunction with glucoamylases to speed up a conversion or very slightly increase RDF. Terminal RDFs of 88% are possible under special conditions. Note that when used alone pullulanases can have an interesting impact. They can raise your RDF several percent while keeping the sugar profile similar to a natural maltose-based profile, thus avoiding the glucose suppression issue while getting a bump in fermentability.

Optimization of Mashing and Beer Filtration: β-Glucanases

If you look back at the discussions around grain modification, you will begin to understand the critical effect high levels of β-glucans and arabinoxylans have on processing your product (Fig. 3). Just getting the product through your straining device can be problematic, at best, if you have borderline or poorly modified grains. Additionally, if you choose, as many do, to add specialty grains that have low levels or are free of the needed enzymes, or are totally lacking in modification from malting, you are probably experiencing problems. These problems can also be translated later into your process and beer flavor. For those that filter their products, the presence of elevated levels of β-glucans and of xylans (as little as 10% equivalent by molecular weight) can result in frustrating filter runs. As pointed out earlier, I believe in a consistent process flow. I want my beer to possess the flavor attributes I choose and not those caused by difficult or delayed runoffs and filtration. Husky, grainy, and oxidized notes are just a few of the issues created by this struggle, a struggle that you can make go away with very little effort. In the market today there are a large number of available β-glucanases, xylanases, and combination products. In addition, you may run into products labeled as pentosanases and cellulases. These are just different examples of what can be used. The pentosanases are technically xylanases, which I refer to specifically later, and the cellulases are typically blends of β-glucanases and xylanases, which I also refer to later. What your problems are, or what your objectives are, will play into your decision on which to use. The most popular product to use is just a β-glucanase. Most brewers who use these do so to remove the variation they are getting with their malt. If you are not paying for highly controlled malt processing, you may be getting variability in your grains. By adding a small amount of typical β-glucanase, much of this variation can go away. Additionally, you may see a yield increase that easily offsets the cost of the enzyme.

Optimization of Mashing and Beer Filtration: Xylanases

Getting a little more specific, we will now look at xylanases, more exactly arabinoxylanases. As you will recall, arabinoxylans are copolymers of two pentose sugars, arabinose and xylose. This molecule is an integral part of the structural makeup of the grain, closely associated with the β-glucans in this structure. In most modification discussions and papers, little credit is given to arabinoxylans, when in fact these are the evil brothers of the β-glucans. The issues with arabinoxylans come with the modification of the grain as well as the use of specialty unmalted grains that are high in arabinoxylans, such as wheat and rye. Variability in modification of the grains during malting is normally the cause of arabinoxylan issues arising from malting. The addition of a xylanase can be beneficial to your process if you are seeing issues derived from the presence of xylans, but you need to approach any hydrolysis of arabinoxy-

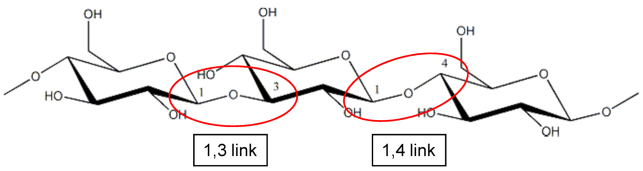

Figure 3. β-Glucan molecule. Image courtesy of DuPont.

lans with great caution. We discussed earlier the close association of ferulic acid with this molecule. Notably, its digestion with enzymes can yield higher than normal levels of free ferulic acid in your wort and then, depending upon your process, issues with taste from the conversion product, 4VG. When you consider the breakdown of xylans with commercial enzymes, consider that the ferulic acid is more closely tied to the water-soluble portion of the xylans. If you select the wrong enzyme, hydrolyzing major fractions of non-water-soluble xylans can be problematic. Lastly, I mentioned wheat and rye specifically here because of their high xylan content. This is also true of unmalted barley, but wheat and rye possess a protein that suppresses the effectiveness of most commercial xylanases. There are a few commercial enzymes that can be effectively used under these conditions. Understanding that this can be an issue will help you find the right solution because you can ask the right questions. Using whatever is on the shelf will get you into trouble.

Optimization of Mashing and Beer Filtration: Combination

Today, most commercial β-glucanase/xylanase products come with some mixed activity. Because issues typically arise from variability in modification, these combination enzymes are most useful. Additionally, when working with unmodified grains, these enzymes will open up the grain matrix to allow full exposure of the grains to the brewing processes. In all cases, caution needs to be taken to avoid a 4VG issue. Your process and hot delays will determine if a problem can or will occur.

Cost-Effective Cereal Cooking: Amylases Again

If you are cooking cereal grains such as corn and rice, the use of exogenous amylases may be beneficial. The objective in cooking is to gelatinize and then liquefy these grains to expose the starch for enzymatic attack for conversion to fermentable sugars. Looks fairly easy—add some malt and give it a boil to blow up the starch granules. However, if you are working with poorly modified malt or a large component of unmalted grains, getting enough enzymatic power into the cooker can be a problem. This problem can be translated into taste impacts from boiled grain as well as a loss of enzymatic power for your conversion, because you boiled the grain portion placed in the cooker and killed all of the enzymes. If you back away from the malt addition to your cooker, you can substitute the use of commercially derived amylases. The benefits are obvious to the rest of your process, but the hidden benefit is yield. By using a mid- and high-temperature blended amylase you can liquefy this grain even without boiling (energy savings). You will need to get the mash to above 90°C, but a boil is not required. Additionally, the traditional enzymes would be fully destroyed soon after you need them, because the gelatinization takes place very close to their deactivation temperature. With the blended amylases, the high-temperature amylase takes over and will continue to function though most of the boil, freeing up additional starch (increased extract).

Cost-Effective Adjunct or Malt: Proteases and Peptidases

Typically, if you are going to have a problem with FAN levels in your wort you are either stretching the limits of your processes or you are using non-protein-bearing materials that are diluting the materials that bring you the FAN. In this case, you may need to add a protease or peptidase to digest some of the unused protein down to amino acids for your yeast. Proteases can be problematic in that they can reduce the foam characteristics of your beers. However, if you are careful to use only what you need to achieve the results required for your fermentations, you can normally avoid issues. The problems with proteases come with overdosing. They have traditionally been given a black eye because of the use of proteases as chill stabilizers. In this use, the enzymes were left to work unrestricted for long periods of time, and they were typically overdosed for effect. Cautious use in mashing can have the needed impact to stabilize your process, should you be short on available FAN.

Optimal or Improved Fermentation Control: α-Acetolactate Decarboxylase

As part of a normal fermentation cycle, fermenting beers generates diacetyl. The diacetyl comes from a side reaction of yeast converting available amino acids for its own use. From this reaction, the resultant α-acetolactic acid is expelled from the yeast as a by-product. In solution, this compound undergoes spontaneous oxidative decarboxylation, taking the compound to diacetyl. Under normal conditions, yeast will take the diacetyl back into the cell and, via an energy-reclaiming processes, an enzymatic transition using a reductase will convert the diacetyl to acetoin. Again, the yeast cell will expel the acetoin, but the flavor impacts of acetoin are much less than diacetyl. There are techniques that can be applied from FAN levels to air content and so on that can effectively manage the creation and later uptake of diacetyl. However, if you are experiencing control issues that cannot be dealt with in any other way, there is an enzyme known as α-acetolactate decarboxylase that is added after the brewhouse. This enzyme will grab onto the expelled α-acetolactic acid and convert it directly to acetoin, while still in solution, outside the yeast cell. What this does is allow you some freedom in fermenting capacity as well as some control of diacetyl, if necessary, as you stretch the limits of your processes through the addition of nonmodified grains. I must caution you that this enzyme is not a solution for high diacetyl in a product. It will not have any effect on already formed diacetyl.

Specialty Grains: Various Enzymes

When we look at enzyme solutions for use with specialty grains, we need to ask some questions around how much, what materials, and what portion of the grain bill remaining is good quality well-modified malt. In simple terms, the industry groups percentages of specialty grains into ranges. For example, we look at 0–10, 10–15, 15–25, 25–40, 40–60, and >60% (to include 100%). When using well-modified malt, the 0–10% range offers few issues. Sometimes you may need a little help with the β-glucan and xylans, as discussed earlier, but no other issue should arise. As we move up the scale, you are looking at a need to add more and more aggressive enzyme complexes to gain access to the nonmodified grains and to keep the processes running free of delays. The higher you go, the less FAN you have available, because you are diluting the modified malt portion of your grain bill. Again, if you have well-modified malt you can get to upward of 50% substitution without the need of a protease. As you increase your addition of specialty grains, the need for β-glucanases and xylanases can go higher and higher. As you increase these ratios, you move away from simple blended β-glucanase and xylanase products to products that have more of the same but with varying temperature sensi-

tivities. In that manner, the enzymes provide a broad attack on the β-glucan and xylans. Therefore, in looking at the 10–15% addition range, the more basic solutions mentioned earlier are appropriate. Sometimes the more simple solutions can be applied to the 15–25% range as well; it may be just a matter of dosage rate. However, once you go above 25%, the problems can be challenging. At this point you might see a need for an amylase to be added as well, but this is a matter for close review. Commercial enzyme suppliers normally have complex blends of β-glucanase, xylanase, pentosanases, amylases, proteases, and peptidases. Sometimes you need all of these and sometimes you do not. My recommendation to you is to approach these issues with your eyes wide open and with some expertise in your pocket. Remember, if you are using wheat or rye, the game changes a little. Lastly, as you use these unmalted grains in high percentages, often the filterability of your straining device suffers. Sometimes it is an effect caused by an increase in β-glucans and xylans, and sometimes it a loss of husk materials. As you venture down this road, consider the use of filter aids in your mash as well. Addition of rice hulls, for example, can greatly improve your run-off and wort characteristics.

Improved Haze Control: Proline-Specific Endoprotease

A recent introduction to the family of commercial enzymes is an enzyme that can be added postfermentation for the purpose of chill haze stabilization. This highly specific protease attacks a specific sequence of amino acids that tends to be proline-rich. Traditionally, proline-rich protein fractions have been tied to chill haze issues. These proline-rich fragments form the protein–polyphenol complexes associated with chill haze. By breaking down these proline-rich fragments, brewers have been able to effectively improve the chill haze stability of beers. The enzyme specifically catalyzes a carboxyl-terminal hydrolysis of proline, thus rendering it unavailable to assist with building these protein–polyphenol haze complexes. As an aside, there has been some discussion regarding this enzyme's ability to also reduce, at least analytically, a portion of gluten in beer, thus rendering a product analytically low-gluten or gluten-free. The actual effect of this enzyme to change the product so it does not impact celiacs is under evaluation.

Summary

By now it should be obvious that the enzyme framework in brewing far exceeds the basics of α- and β-amylase. Although the most important enzymes related to the process of brewing, they are shadowed by the magnitude of the remaining enzymes mentioned here. It should be noted again that what is mentioned here is not an all-encompassing review of what enzymes influence the process; these are just the main ones.

It should also be clear from a review that malting is not simply a process of growing barley. It is a controlled process of influencing natural enzymes along the way. These enzymes provide the brewer with the tools to make beer. It is also easy to see that what happens in malting translates well to brewing and beer. The brewing process takes consideration of all that happens in malting and more. The small effects of other enzymes can have a devastating effect on products. Awareness is the driver to making a great beer.

When all else fails or there is a need to stretch the limits of the process beyond what can be done naturally, there are tools at the brewer's disposal: commercial enzymes. As noted within the context here, even these enzymes need to be approached with your eyes open. As with almost everything done in brewing, there are consequences to each move made in a process. It is the balance that makes the best beers. This balance is gained through greater understanding of as many of the causes and effects as possible. From this awareness we can relate to process change and make the best decisions for products.

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