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# **Enzymes in Brewing Series, Part 3 - Commercial Sources** Preface

In the last two editions (Issues 4 & 5, Volume 2) we 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 into this discussion we will begin with many of the same enzymes seen in the past two articles, but these are now coming from non-malt sources. Typically these are derived in fermentations similar to brewing culture operations but the organisms producing the enzymes are either bacterium or fungi that is not yeast. Additionally as I progress through each of these enzyme groups I will not reflect specific benefits of using one company's product verses the other. It should be noted that often they are extremely similar, but sometimes they are very different, primarily because of the various host organisms chosen to make the enzymes. Often, side activities of beneficial and sometimes non-beneficial enzymes, that arrive with the production of the principle enzyme, come into play and can offer a wide variety of outcomes when using a product. So, when looking at these products, it is wise to ask about these side activities as well as conducting bench-top mashes for evaluation of outcome.

Also, within this discussion I will not reference dosing rates as these too will vary from product to product. As I will state below, I have used many of these and are experienced in their strengths, weaknesses and variations. If you find that you are interested in using a specific type, I would gladly run through the options with you and give you some of this background. Lastly, for the purpose of keeping the article with some range of reasonable size I will not take on the discussion of the range of products to use if you looking at very high additions or specific brewing with specialized grains like sorghum or greater than 50% unmalted grains. This can get pretty tricky and complex. Again, if this is your interest we can discuss that over a beer. We will however, touch upon the use of barley, rye, and wheat as these additions can pose significant challenges to any brewer.

Issue 6, Volume 2

7/8/13



The choices you make to help your brewing process, by using these enzymes, can be greatly influenced by the knowledge of grain specifics.

## Introduction

Most commercial enzyme companies break their products into several basic areas. The first two major groups deal with Attenuation Control and Optimization of Mashing and Beer Filtration. The next major group often is associated with Cost Effective Cereal Cooking. However, many of these enzymes can be used in improving your attenuation control. Sometimes enzyme classifications may roll over on one area or the next. Don't concern yourself with this. Once you understand the basics of each specific enzyme, you can then use this knowledge to cross these boundaries. The next groups get more specific. These groups fall into categories that are often called Cost Effective Adjunct and Malt opportunities, Optimal or Improved Fermentation control, Special 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 you do today, thus stopping any changes driven by their activity.

### **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/or amyloglucosidases, and limit dextrinases and/or pullulanases. Let's begin with the amylases.

### **Attenuation Control- Maltose Producing Amylase**

As per our previous discussions we looked at the natural occurrence of alpha and beta amylases in malt. These two enzymes are available in several forms commercially as well. As we walk through these, consider your processes as there may be several reasons to consider these use of these enzymes in that they can increase the rate of reaction, increase your yield and help you attain your end alcohol targets, should you be using either poorly modified malts or cereal grains lacking in enzyme power. To begin, Beta Amylases are hard to come by. Typically this enzyme is only available as a



malt extract and is not typically used as a commercially derived product because of its cost and the availability of other products. There is currently only one other alternative to beta amylase and this is a widely used product; fungal alpha amylase (FAA). Although this product is very temperature sensitive, it does work very similar to malt beta amylase in producing maltose as it digests starch. There has been industry discussion to look into this area, but as of yet there is not a commercially available alternative. If you need to tune a conversion the FAA can help you maintain a maltose-based wort, thus avoiding glucose suppression issues with high-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 below. However, if greater detail is needed, I am available for a discussion, or we can look at <del>as</del> another article if interest dictates.

# **Attenuation Control- Alpha Amylases**

Moving into the alpha amylases, there is a vast range of these. Some that are available will have similar sensitivities to temperature and pH as the malt alpha amylase and you can look at these as enhancements. However, there are others that are mid and high to very high temperature amylases that were developed primarily for the syrup industry. These products come as blends or individual products. For brewing I would recommend a mid to high-range blended product as they will reduce your viscosity quickly at lower temperatures and at the high temperature the enzyme will provide extract, etc. I will say more about that below. However, for attenuation control, 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 glucoamylases or GA's. You may on occasion hear these referred to as amyloglucosidases (AMG's), but for the purpose of this discussion you can consider these to be the same. Glucoamylases attack starch molecules from their non-reducing 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 a 1-6 branch point. When used with well-modified malt (an ample supply of alpha 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 non-special circumstances, RDF's over 80% are without event. Values approaching 85% are fairly easy with some special considerations and values as high at 87% have been achieved in all malt mashes. I mentioned glucose suppression above and typically issues of such can come into play with the use of GA's. In the discussion here, our wish is to convert most of the sugars to glucose. If you do this and get most, perhaps 70% of it over to glucose, you will normally not see an issue. The glucose suppression issue comes with using small amounts of GA as an enhancement to an RDF that is naturally based. 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 RDF's just-under 80%. It's when you want to get just a little more that you can experience a problem. Once you convert some of the sugars to glucose you begin to enter a nomans-land of 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 make the decision to either continuing to ferment, or to shut down. If the fermentation shuts down, it can get interesting. As I said earlier, this is a topic within itself. Your interest will determine if we look into it. See the end of the article for my contact information.

# Attenuation Control- Limit Dextrinases/Pullulanases

One of the more interesting group enzymes available is the limit dextrinases (also known as pullulanases). For the sake of consistency I will refer to them as pullulanases. These enzymes possess the ability to break the 1-6 branch points in starch. One might think this is really cool and that this enzyme will have a major impact on my RDF. However, in reality, due to the normal activity of the alpha amylases, one does not see a major impact. But, one can use these either in conjunction with GA's to speed up a conversion or very slightly increase an RDF. Terminal RDF's of 88% are possible under very special conditions. Secondly, when used alone, they have a very interesting impact. They can raise your RDF several percent while keeping the sugar profile very 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- Beta Glucanases**

If you look back at the discussions around grain modification in the first of this series of discussions, you will begin to understand the critical effect high levels of beta glucans and arabinoxylans have on processing your product. 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 of you do, to add specialty grains that are low or free of the needed enzymes, or lacking totally in modification from malting, you are experiencing problems. These problems can also be translated later into your process as well. I know most of you do not filter your products, but for those that do, the presence of elevated levels of beta glucans and, xylans, as little as 10% equivalent by molecular weight, can result in very frustrating filter runs. As I pointed out earlier, I believe in a consistent process flow. I believe in this because I want my beer to possess the flavor attributes I want for it and not those caused by difficult or delayed runoffs and/or filtration. Husky, grainy and oxidized notes are just a few of the issues created by this struggle, a struggle that can be made to go away with very little effort. In the market today there are a large number of available beta glucanases, xylanases, and combination products. In addition you may run into products labeled as pentosanases and cellulases. These are just different examples that can be used. The pentosanases are technically xylanases, which I refer to specifically below, and the cellulases are typically blends of beta glucanases and xylanases, which I also refer to below. Your problems and/or your objectives will play into your decision on which to use. The most popular product to use is just a beta glucanase. Most brewers who use these enzymes use them to remove the variation they are getting with their malt. If you are not paying for highly controlled malt processing, you are getting variability in the grains you are buying, without question. By adding a very small amount of typical beta glucanases, much of this variation can go away. Additionally you will 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 co-polymers of two pentose sugars: arabinose and xylose. This molecule is an integral part of the structural makeup of the grain, closely associated with the beta 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 beta 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, like wheat and rye. Variability in modification of the grains during malting is normally the case of arabinoxylan issues arising from malting. The issue for you is what to do about it? The addition of a xylanase can be very beneficial to your issue, but you need to approach any hydrolysis of arabinoxylans with great caution. As you recall we discussed the close association of ferulic acid with this molecule. Notably, it's 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: 4 VG. But all is not lost. One company has recently introduced discussions around a very specific xylanase that leaves this fraction of the arabinoxylans alone. Targeted enzymes are the next great wave of opportunity. Lastly, I mention wheat and rye specifically here because of their very high xylan content. This is also true of unmalted barley, however, wheat and rye possess a protein that suppresses the attack of most commercial xylanases. As with the targeted xylanase, one company has a solution, an enzyme that is not suppressed by this protein. Others are also looking into solutions that will address this specific problem. Of note; it is not that other enzymes available today will not work; you just have to dose upwards of 5 times the amount of the non-suppressed version.

### **Optimization of Mashing and Beer Filtration- Combination**

Today, most commercial beta glucanase/xylanase products come with some mixed activity. Because the issues arise from the lack of or variability of 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 4 VG issue. Your process and hot delays will determine if a problem can or will occur.

#### **Cost Effective Cereal Cooking- Amylases Again**

An issue that most of you do not deal with but is worth mentioning is the cooking of cereal grains, like corn and rice. The objective is to gelatinize and then liquefy these grains to expose the starch for enzymatic attack for the conversion of these 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 loss of enzymatic power for your conversion because you boiled the grain potion 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 degrees Centigrade, but a boil is not required. Additionally the traditional enzymes would be fully destroyed soon after you need them as 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 less expensive materials that are diluting the materials that bring you the FAN. In this case, you may need to add a protease and/or a peptidase to digest some of the unused protein down to amino acids for your yeast. Yes, proteases can be problematic in that they can reduce the foam characteristics of your beers. But, 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 over-dosing. They have traditionally been given a black eye from the use of proteases as chill stabilizers. In this use, the products were left to work unrestricted for long periods of time, and they were typically over-dosed for effect. Cautious use in mashing can have the needed impact to stabilize your process, should you be short on available Free Amino Nitrogen.

### **Optimal or Improved Fermentation Control- Alpha-acetolactate decarboxylase**

Wow, what a mouthful. In review, as you ferment your beers, this process generates diacetyl. The diacetyl comes from a side reaction of your yeast converting available amino acids for its own use. From this reaction, the resultant alpha-acetolactic acid is expelled from the yeast as a byproduct. In solution this compound undergoes



spontaneous oxidative decarboxylation, taking the compound to diacetyl. Under normal yeast life experiences it will take the diacetyl back into the cell and, via an energy reclaiming process, 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. In the real world this is controllable to where you want it. There are process techniques that can be applied from FAN to air content, etc. Effective techniques that can balance yeast growth and D levels are obvious opportunities for greater discussion. However, there is one company that supplies an alpha-acetolactate decarboxylase that is added post brewhouse. This enzyme will grab onto the expelled alpha-acetolactic acid and convert it directly to acetoin, while still in solution, outside the yeast cell. What this does for you is allow you some freedom in fermenting capacity as well as some control of D, if necessary, as you stretch the limits of your processes through the addition of non-modified grains. I must caution you that this enzyme is not a solution for high D in a product. It will not have any effect on already formed diacetyl.

# **Specialty Grains- Various Enzymes**

When we look at enzyme solutions for the use of 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, we group percentages of specialty grains into ranges. For example, we look at 0-10%, 10-15%, 15-25%, 25-40%, 40-60% and over 60% to include 100%. When using well-modified malt, the 0-10% range offers little issue.

Sometime you may need a little help with the beta glucan and xylans as discussed above. But for certain, 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 non-modified grains. 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 upwards of a 50% substitution without the need of a protease. As you increase your addition of specialty grains the need for Beta glucanases and xylanases goes higher and higher. So, as you increase these ratios you move away from simple blended beta glucanase and xylanase products to products that have more of the same but with varying temperature sensitivities.



In that manner, the enzymes provide a broad attack on the beta glucan and xylans. Therefore, in looking at the 10-15% addition, the more basic solutions mentioned above are appropriate.

Sometimes simpler solutions can be applied to the 15-25% range; it may be just a matter of dosage rate. However, once you go above 25%, the problems can be very difficult. At this point we might see a need for an Amylase to be added as well, but this is a matter of close review. Commercial enzyme suppliers normally have complex blends of beta glucanase, xylanase, pentosanases, amylases, proteases and peptidases. Sometimes you need all of these and sometimes you don't. 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 and/or rye, the game changes a little. Your supplier options really drop to one. 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 beta glucans and xylans and sometimes it is 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 Endo Protease

Over the past few years one company has been introducing, and at the same time improving, an enzyme that can be added post fermentation for the purpose of chill haze stabilization. Today, the traditional methods of silica gel; PVPP, etc. are mechanical means of removing, supposedly, haze active proteins or polyphenols. What one company has determined is that the majority of all the haze forming proteins in the protein/polyphenol haze complex are rich in proline (an amino acid) sequences. What they have developed is an enzyme that specifically catalyzes a carboxyl-terminal hydrolysis of proline, thus rendering it unavailable to assist with building these protein/polyphenol haze complexes. It is not my place to sell you a product, but this looks interesting as an alternative to mechanical separation.



## **PRO Engineering Services**

As we walk away from this series one should be able to see the critical nature of brewhouse control. This is not only control of the obvious, temperature, but time as well. Additionally agitation, insulation, pumping capacity, heating and cooling capacity all play roles in your control of what you are trying to do when you challenge yourself with something different. Within the context of this article, sometimes the use of enzymes requires the addition of proportioning addition equipment and batch scaling. I'm not the engineer and cannot offer to you these services. However, your host for these articles can. PRO Engineering and Manufacturing Inc. can offer you a wide range of fully scalable and integrated solutions to your "opportunities". Please consider them to partner with you and help you grow with new possibilities.

#### **Next Edition?**

This is the last edition in this three part series. Within the series I made reference to opportunities for greater discussion. Examples of these opportunities could be **Malting Control in Detail, Specialty Grains, FAN in Detail, Specialty Mashing, Diacetyl Control and perhaps The Other Enzymes.** I hope you enjoyed this series of three issues on enzymes in brewing and found some tools that you can use to help you in your processes. As always, I am open to your thoughts and questions, please feel free to share. Contact me at the address below.

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Ed Michalski (left) with brother Dave, checking specs for a customer

If you need brewing equipment repaired, or re-engineered to work better, faster or more cost-effectively, contact Ed Michalski, CEO, at PRO Engineering and Manufacturing, Inc.

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