ENZYMES ARE CATALYSTS for specific biochemical reactions. Almost all enzymes are large protein molecules. The overwhelming majority of biological processes need enzymes in order to occur at a meaningful rate. Their ability to work depends upon their shape. Like any protein, an enzyme can be denatured, by heat or acidity, for example, so that it no longer works.

In winemaking, several enzymes have been approved for use in wine by the Food and Drug Administration (FDA) and Alcohol and Tobacco Tax and Trade Bureau (TTB). For the purposes of this article, we will call these enzymes “Enological Enzymes.” It should be noted that enological enzymes are distinct from the analytical enzymes one might use in a winery lab as part of a routine analysis for glucose-fructose, for example.

ENZYMES AND ENZYME BLENDS
Essentially all of the “enzymes” sold for winemaking are actually complex cocktails of several enzymes. In this article, the term “enzyme” will refer to single enzymes with a particular activity (such as pectinase). The term “enzyme-blends” will be used as a catch-all term for any enological “enzymes” one can purchase, whether it contains only one or several enzymes.

To further confuse the matter, enzymes are usually defined by their activity rather than their exact chemical structures or even the biological source from which they are derived. As we’ll see below, this only makes a difference in a couple of individual cases.

IT’S ALL ABOUT POLYSACCHARIDES
All enological enzymes function by cleaving the bonds between the individual sugar-units in polysaccharides. This is because polysaccharides make up the structure of the grape cells.

For example, cellulose is a polysaccharide of anywhere from 7,000 to 15,000 linked glucose molecules. Hemicellulose is any one of several polysaccharides consisting mainly of glucose and xylose with a leavening of arabinose, galactose, mannose and/or rhamnose. Pectins are polysaccharides of galactose (actually the oxidized form: D-galacturonic acid), possibly with other sugars like rhamnose, arabinose, xylose, etc. forming sidechains.

DEE AND ELL, ALPHA AND BETA
When one starts discussing polysaccharides and enzymes, one is forced into a certain amount of very specific chemical and biochemical terminology. The D- and L- prefixes like one finds in “D-glucose” or “L-fructose,” for example, refer to the stereochemistry. D-glucose and L-glucose are chemically identical, but they are mirror images of each other. Since almost all naturally occurring sugars are D- sugars, we generally don’t bother to explicitly list the D-; however, enzymes are the reason almost all natural sugars are D- sugars.

The reason I’m mentioning D-/L- stereochemistry here is to emphasize that enzymes are extremely sensitive to substrate stereochemistry. If one were to chemically synthesize glucose, the result would be an equal amount of L-glucose and D-glucose. However, if one uses a living system, like a plant, to make glucose, all one gets is D-glucose.

Perhaps more pertinent to the subject at hand is the α- and β- prefixes used to describe the stereochemistry of glycosidic bonds in cyclical sugars like glucose. Alpha-glycosidic bonds are out of the ring plane while beta-glycosidic bonds lie in the same plane as the ring. Examples are starch, which is an β-linked polysaccharide of glucose, and cellulose, which is a β-linked glucose polysaccharide. In general, an enzyme that cleaves β-glycosidic bonds won’t cleave α-glycosidic bonds and vice versa.

HOW ENOLOGICAL ENZYMES ARE USED
Enological enzymes can be used at several stages in winemaking. These uses tend to fall into just a few categories: extraction, clarification, filtration and the inhibition of gram-positive bacteria. With the exception of lysozyme, which is used only for the inhibition of gram-positive bacteria, there is a great deal of overlap between the enzymes.

Filtration: Pretty much any enzyme blend used for clarification can be used as an aid for filtration. Like clarification enzymes, filtration enzymes are usually a blend of pectinase and cellulase, hemicellulase, β-glucanase, amylase and protease. Since, in theory, the solids have been settled out prior to filtration, fairly aggressive enzymes (see below) derived from Asperillus niger can be used.

Filtration enzymes are used to break up soluble pectins and polysaccharide colloids. This makes the wines easier to filter. Note, however, that colloids will reform given enough time, so it doesn’t do any good if one delays filtration too long after adding enzymes.

INHIBITION OF GRAM-POSITIVE BACTERIA
Lysozyme was first isolated in 1922 by Alexander Fleming. In winemaking, it is only used to inhibit gram-positive bacteria, specifically lactic acid bacteria (LAB). It has no real inhibitory effect on gram-negative bacteria like aceto-bacter or on anaerobic spoilage yeast like Brettanomyces or film yeasts like Pichia spp., Candida spp. and Hansenula spp. Despite a limited number of winemaking applications, lysozyme functions in a manner fairly similar to all other enological enzymes.

Product Review: Enological Enzymes
A winemaking tool for extraction, settling and clarification

Curtis Phillips
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Stelvin® twist tops. It’s a matter of taste.
SPECIFIC ENZYMES
Pectinase (AKA polygalacturonase): Pectinase breaks down pectin. Of most polysaccharides, pectin is not a single chemical. The term pectins describes a broad family of soluble heterogenous polysaccharides. They form part of the structure of primary cell walls in most non-woody plants. Pectins are also found between the cells, in the middle lamella where they help to bind the cells together. Pectinase breaks down pectin by the random hydrolysis of (1-4)-alpha-D-galactosiduronic linkages in pectate and other galacturonans.

Cellulase: Cellulose is a polysaccharide of glucose. Cellulase breaks down cellulose to either simple glucose or into glucose-disaccharide.

Hemicellulase: Hemicellulase breaks down hemicellulose. Hemicelluloses are polysaccharides composed of a broad range of simple sugar monomers including glucose, xylose, arabinose, galactose, mannose and/or rhamnose. Hemicellulose chains (500 to 3,000 sugar units) are much shorter than cellulose chains (7,000 to 15,000 sugar units).

Glucanase: A glucanase is any enzyme that breaks down glucans. Glucans are polysaccharides of glucose. Dextran, glycogen and starch are all examples of alpha-glucans while cellulose is an example of a beta-glucan.

Glycosidase: Glycosidases are hydrolyses which attack glycosidic bonds in carbohydrates, glycoproteins and glycolipids. The glycosidases are not highly specific. Usually they distinguish only the type of bond, such as O- or N-glycosidic and its configuration (alpha or beta).

\[ \beta\text{-glucosidase: } \] Beta-glucosidase cleaves the beta-D-glucosides.

Rhamnosidase, Apiosidase and Arabinofuranosidase: Apiosidase, arabinofuranosidase and rhamnosidase are used to release terpenes.

Lysozyme: In winemaking, lysozyme is used to inhibit the growth of gram-positive bacteria, especially lactic acid bacteria (LAB).

USING SO-CALLED “AGGRESSIVE ENZYMES”
Scott Laboratories puts a pretty stern warning on their Scottzyme KS: “Warning: Never use Scottzyme KS before pressing (i.e., at the crusher for whites, or before or during red fermentation). Scottzyme KS has very aggressive enzymatic activities that will destroy skins and create too many fine solids.”

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EVERYTHING YOU NEED TO MAKE GREAT WINE.

Yokayo Wine Company invites all segments of the industry to make their wine at one of Northern California’s finest custom crush facilities. Yokayo is a new kind of operation, ready to meet the business needs of today’s winemaker.

Not only do we have a great winemaking staff, we also provide you with expert compliance services, so you can forget about red tape and concentrate on red wine, instead. Or white. Or rosé. With a wide selection of fruit available, we’re ready to help you craft whatever great wine you wish to make.

Yokayo Wine Company invites all segments of the industry to make their wine at one of Northern California’s finest custom crush facilities. Yokayo is a new kind of operation, ready to meet the business needs of today’s winemaker.

Welcome to the next generation in wine yeast

Maurivin is proud to introduce its new range of Next Generation wine yeast. This includes the world’s first yeast that eliminates reductive characters in winemaking. And our exciting new range of hybrid strains for enhanced complexity and increased mouthfeel. Working with the world’s leading research institutes, Maurivin’s Next Generation yeast aims to maximize your wines’ varietal aromas and flavors.

Maurivin. Setting the new benchmark in wine yeast.
The reason for the warning is that the enzymes in Scottzyme KS are derived from Aspergillus niger. A. niger is a spoilage fungus, and it produces some enzymes that are very good at breaking down the cellulose, hemicellulose and pectins in grape berry cell walls. In the winery, these enzymes are used as a filter aid by breaking up glucans-produced botrytis. One should avoid using any enzymes from A. niger prior to pressing.

Enzymes can only extract what’s already in the grape skins. However, extraction enzymes can help speed things along.

Gusmer offers winemakers the best in enzymes, tannins and granular oaks for use during crushing and fermentation. Enzymes, like Vinozym® Vintage FCE, pure grape tannins from Ferco and Gusmer’s Oak Avantage® granular oak all help to achieve the deep red color, improved flavors and aromas, and lush mouth feel so desired in red wines.

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ENZYMES DERIVED FROM TRICHORDERMA HARZIANUM
Laffort Oenologie has introduced two enzyme blends containing β-glucanase derived from Trichorderma harzianum called Extralyse and Filtrozym. The FDA has approved this β-glucanase for use in wine, but at the time this article was written, it is still undergoing approval with the TTB. This means that any winery desiring to use these enzymes needs to file a request to do so with the TTB before using them. In addition, the winery is required to maintain records of the treatment. See 27 CFR 24.250 and 27 CFR 24.301(h) for more information.

“OFF LABEL” USES
To my mind, the best use for color-extraction enzymes is actually as a clarifying agent for white wines.

WINEMAKING WITH ENZYMES
These days, most enological enzymes are sold as direct-addition powders. A few are sold as “live enzymes” in liquid solution. In all cases, I suggest that users read the manufacturer’s instructions carefully as the concentration and activity can vary substantially with recommended doses ranging from 5 g/hL (0.7 oz. per 100 gal.) up to 10 times that amount depending on the particular enzyme-blend used and particular application for which it is used.

WHAT HAPPENS AFTER?
Since enzymes are proteins, they will naturally denature and lose their activity over time. Also, wine is a pretty harsh environment, and enzymes are sensitive to temperature, acidity and ethanol. Most denatured enological enzymes will precipitate out of solution if the wine is given enough time or if the wine is fined with bentonite.

MUSCATS, RIESLINGS, AROMATIC WHITES
There are two key aspects to using enzymes for aromatic whites. The first is getting the juice out of the berry. Winemaker Linda Trotta noted that, “At Gundlach Bundschu, I routinely used an enzyme with Gewürztraminer and Sauvignon Blanc, primarily to aid in settling. Lafazyme Press (Scott Labs) is what we found worked best for our situation. We would add it to the fruit as it went into the press. It was
particularly helpful on reducing gross lees in the portion of Gewürztraminer that we cold-soaked on skins overnight. In general, [it] increased free run juice volume, decreased the quantity of gross lees; and since its activity lowers phenolic compounds (including limiting vinyl phenol production), in my assessment the resultant fruit expression is “fresher.”

The second is releasing bound terpenes since the characteristic varietal aroma of most “aromatic” white varietals derives from these compounds. To aid this process using enological enzymes, an enzyme-blend containing pectinase can be added at the crusher. In addition, enzyme-blends containing pectinase and β-glucosidase can be added after fermentation. Beta-glucosidase is inhibited by the presence of simple sugars so such blends don’t really do any good if one adds any before the wine is below 0.5 percent residual sugar.

**LOW-COLOR REDS (EXTRACTION AND STABILITY)**

**EXAMPLE:**

**Lodi Zin and Merlot**

Low color reds produce a quandary. Enzymes can only extract what’s already in the grape skins. However, extraction enzymes can help speed things along for quick-to-market reds. The main drawback is that they tend to make the cap really sludgy and hard to press. I recommend that anyone using enzymes on reds keeps a good supply of rice hulls on hand as they become indispensable when pressing. When mixed with the must in the press, the rice hulls provide enough physical structure so that the wine can be separated from the pomace. Otherwise, the sludgy cap just clogs the drain holes and traps the bulk of the wine in the press.

**SETTLING, CLARIFICATION AND FILTRATION**

Enzyme-blends for settling and clarification should contain pectinase. The mechanism for clarification is kind of interesting. Although the overall charge of a pectin molecule is neutral, like most big organic molecules it has some polarity thanks to the presence of oxygen and/or nitrogen, in its structure. This gives the pectin molecules regions that have a positive polarity and corresponding areas of negative polarity (also called areas of higher electron density). If the pectin is soluble, it forms a kind of pectin clump where the areas that are more negative are on the outside and those that are more positive are on the inside. It appears that as the big, but soluble, pectins are cut into smaller pieces by the pectinase, these areas of positive polarity are exposed and thereby are able to form a weak type ionic bond with the negative polarity part of another pectin. These bonds are much weaker than the covalent bonds formed by the condensation reactions that polymerize polysaccharides, but they are strong enough to cause the pectins to flocculate and drop out of solution.

**FILTRATION OF BOTRYTIZED DESSERT WINES**

Botrytized wines are a case of “more glucans.” The “noble rot” Botrytis cinerea produces glucans which can...
### Enzyme Types

<table>
<thead>
<tr>
<th>Enzyme Type</th>
<th>Broad Activity Type:</th>
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<tr>
<td><strong>Pectinase</strong></td>
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<td><strong>Lysozyme</strong></td>
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*Only use after fermentation*

**Some β-Glucosidase-Pectinase blends used for clarifying juice & wine from grapes infected with Botrytis cinerea are derived from Trichoderma sp. Wines produced using these enzymes require a special filing with the TTB.**

**Some β-Glucosidase-Pectinase blends used for clarification of musts from grape varieties derived from Trichoderma sp. These enzymes require a special filing with the TTB.**

**Specifically used to control lactic acid bacteria (LAB).**

### Enzymological Enzyme Manufacturers

<table>
<thead>
<tr>
<th>Manufacturers</th>
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<th>Telephone</th>
<th>Web</th>
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<td>AB Enzymes</td>
<td>Fort Mill, SC</td>
<td>803-547-7800</td>
<td><a href="http://www.abenzymes.com">www.abenzymes.com</a></td>
<td>Ruhapect, Rishavin</td>
<td>GW Kant, Pacific Coast Chemicals</td>
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<td>ABB</td>
<td>San Francisco, CA</td>
<td>415-824-1525</td>
<td><a href="http://www.abb-group.com">www.abb-group.com</a></td>
<td>Endozyme</td>
<td>American Tartaric Products</td>
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<td>Begerow</td>
<td>Reston, VA</td>
<td>703-673-1160</td>
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<td>Parzenzyme</td>
<td>AO Wilson, Vintners Supply Company</td>
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<td>Chr. Hansen</td>
<td>Fresno, CA</td>
<td>559-485-2692</td>
<td><a href="http://www.chr-hansen.com">www.chr-hansen.com</a></td>
<td>Lactizyme</td>
<td>Guumer Wine Lab</td>
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<td>DSM</td>
<td>Parsippany, NJ</td>
<td>800-662-4478</td>
<td><a href="http://www.dsm.com">www.dsm.com</a></td>
<td>Rapidase, Delzyome</td>
<td>KLR Machines, Guumer Wine Lab</td>
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<td>Erbsloh Geisenheim AG</td>
<td>Geisenheim, Germany</td>
<td>+49-6722-7080</td>
<td><a href="http://www.ersbloch.com">www.ersbloch.com</a></td>
<td>Trenolene</td>
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<td>Essero</td>
<td>Santa Rosa, CA</td>
<td>707-542-2719</td>
<td><a href="http://www.essescousa.com">www.essescousa.com</a></td>
<td>Progoose, Uvazyme</td>
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<td>Kerry</td>
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<td>+353-66-7182000</td>
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</table>
make wines made from botrytis-infected fruit extremely difficult to filter. Aggressive enzymes derived from either Aspergillus niger and/or Trichoderma harzianum seem to yield the greatest benefit in aiding filtration.

A CONTRARIAN VIEW
OF ENZYMES

Ed Sbragia of Sbragia Family Vineyards voiced a cautionary note: “I started making wine the way I do with my dad Gino Sbragia about 55 years ago. When I was old enough to watch—and later to help—I realized how good a winemaker he was. When I went into winemaking, I realized that a lot of what was available was unnecessary. My dad made wine the way his ancestors did. Now we have stainless steel tanks, refrigeration, great barrels, temperature and humidity-controlled cellars.

“Adam, my son, and I do the least possible without letting the wine become oxidized or spoiled. That has served me well at Beringer for 32 years, and it’s doing well at Sbragia Family Vineyards. Good grapes, a little experience and a great winemaking team make, if Mother Nature cooperates, really good wine. We are the keepers of what comes from the vineyard. I want to keep that as pure an expression of the soil, climate and the people who grow the grapes as I can.”

Sbragia’s point is well taken. Enological enzymes are a winemaking tool like any other. In the end, most of our job as winemakers is to not mess up what we get from the vineyard. There is little that enzymes accomplish that one can’t do provided one has enough time and excellent fruit.

That said, one of the primary problems I find in wineries outside the major winegrowing regions is over-oxidation. Although bad winery sanitation and practices contribute to this, mostly this is driven simply by the difference in the scale of the winemaking. Speed isn’t too critical, when one is working on the large scale, if only because it’s hard to oxidize a 50,000-gallon tank of wine. Unfortunately, as the lot size gets smaller, the reverse is true; it’s only a matter of an all-too-short time before a 100-gallon wine lot oxidizes. One of the key advantages of using enzymes is that they allow one to speed up the process a bit.

Anything that cuts down the time between harvest and bottle and thereby reduces the likelihood for unwanted oxidation is a good thing. This is especially the case for white wines, but even red wines from many smaller producers could benefit from a faster winemaking tempo.

Of course this can be taken too far; enzymes can be overused, especially in the context of using extraction enzymes, which can break up the grape solids to the point that it’s hard to clarify the wine. Other than that, there are few downsides other than cost to using enzymes.