SUGAR UTILIZATION

AND IMPORTANCE RELATIVE TO FERMENTATION AND DISTILLERY ETHANOL YIELDS

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Fermentation—as it relates to beer, wine, and liquor production—is often described as *a process carried out by yeast and bacteria in which sugars are converted to ethyl alcohol*. The "sugar" part of that equation is essential for alcohol production and is a significant factor when calculating yield from any particular "feedstock", which is the starting material (or substrate) for fermentation, supplying the sugar to the yeast. Some sugars like sucrose, fructose and glucose found in cane molasses or granulated sugar are readily fermentable and do not require enzymes or other catalysts to break them down prior to uptake by the yeast. Other feedstocks like corn, wheat and other grains have sugar (glucose, a.k.a. dextrose) that is bound together in the complex carbohydrate "starch". In the case of starch, the sugar must first be released by enzymatic activity, heat and other factors before it can be fermented by the yeast to make ethanol. This is why grain-based spirits like bourbon and whiskey involve elaborate cooking strategies to release sugars prior to fermentation. Here we discuss the various factors associated with sugar utilization by yeast during fermentation, and how residual sugars leftover after fermentation can affect ethanol yield. We will also look at starch and other complex carbohydrates, how they can vary from one source to another, how they are broken down (converted) into simple sugars, and how to diagnose issues related to sugar uptake in fermentation.

READILY FERMENTABLE SUGAR SOURCES

In certain feedstocks, including grapes and various fruit, the sugar content is in a form (sucrose, glucose and fructose, for example) that is already available to the yeast for fermentation and production of ethyl alcohol. Other examples of readily fermentable substrates include various forms of cane products like granulated sugar or molasses. The sugar in cane products is sucrose, a disaccharide (or two component sugar) consisting of glucose and fructose subunits. These sugars are readily taken up by the yeast and do not require pre-processing or enzymatic conversion. When fermenting these substrates, sometimes the only other required additions are yeast and water. We generically use "yeast" here to mean the typical brewers/distillers yeast *Saccharomyces cerevisiae*. That is important to note because there are other sugars (lactose, for example) that may require a different yeast (*Kluyveromyces* species, for example) for complete sugar utilization and successful fermentation.

It may be necessary to add additional nutrients when fermenting certain feedstocks like granulated sugar. The reason is that although granulated sugar is full of carbohydrates, it has little of the micro- or macronutrients needed for other cell processes. One of the most likely elements to be limiting in a sugar solution (sugar wash) is nitrogen. However, there can also be other nutrients that are limiting. If you are unsuccessful fermenting a substrate containing readily fermentable sugars by adding only water and yeast, try adding nitrogen to see if that solves the issue. Beverage distilleries typically use food grade di-ammonium phosphate (a.k.a. DAP) when supplementing with nitrogen. A successful fermentation is when all of the sugars are utilized and if the desired amount of ethanol was produced relevant to the starting sugars (typically measured as Brix, Balling and/or Gravity). If supplementing with nitrogen doesn't do the trick, it may be necessary to add more complex nutrients like yeast extract, malt extract or peptone. There are also several available nutrient supplements available on the market. Table 1 shows how fermentation progresses in a solution with only sugar, water and yeast (A) versus adding nitrogen/DAP (B) or

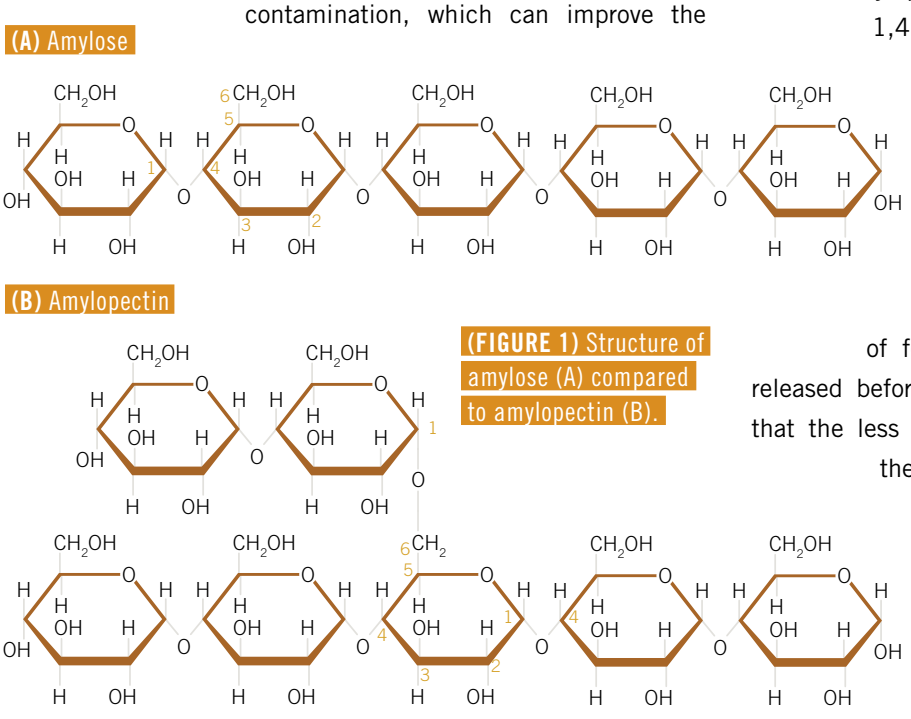
TABLE 1 Fermentation of granulated sugar (18% Brix) with and without supplemental nitrogen and/or complex nutrients. Results shown are at 72 hours.

	FRUCTOSE	GLUCOSE	LACTIC ACID	GLYCEROL	ACETIC ACID	ETHANOL (W/V)
Sugar, water plus yeast	7.102	6.194	0.000	0.228	0.064	0.568
Sugar, water, yeast plus DAP (nitrogen)	5.654	4.495	0.000	0.458	0.143	2.432
Sugar, water, yeast plus DAP, plus complex nutrients	0.076	0.000	0.076	0.922	0.094	8.225
Sugar, water, yeast and complex nutrients (no DAP)	5.432	4.856	0.044	0.459	0.074	2.171

adding nitrogen plus additional complex nutrients (C), or complex nutrients without nitrogen/DAP (D). Clearly, the sugar is nearly unfermentable alone and requires not only additional nitrogen, but also complex nutrients. This is not always the case for other substrates like molasses or other "syrups", which often contain additional nutrients and may require less supplementation than when fermenting sugar alone.

There is a limit to how much sugar can go into solution before osmotic stress (more commonly known as "sugar shock") starts causing issues with the yeast. Thus, there is a "sweet spot" (no pun intended) for sugar concentrations, which depends on the source (granulated sugar, molasses, high fructose corn syrup, etc.), whether there are any toxic factors that may require dilution, and related factors. For molasses and granulated sugar a Brix of between 17-20% is a good place to start and then increase or decrease as necessary to achieve the desired results.

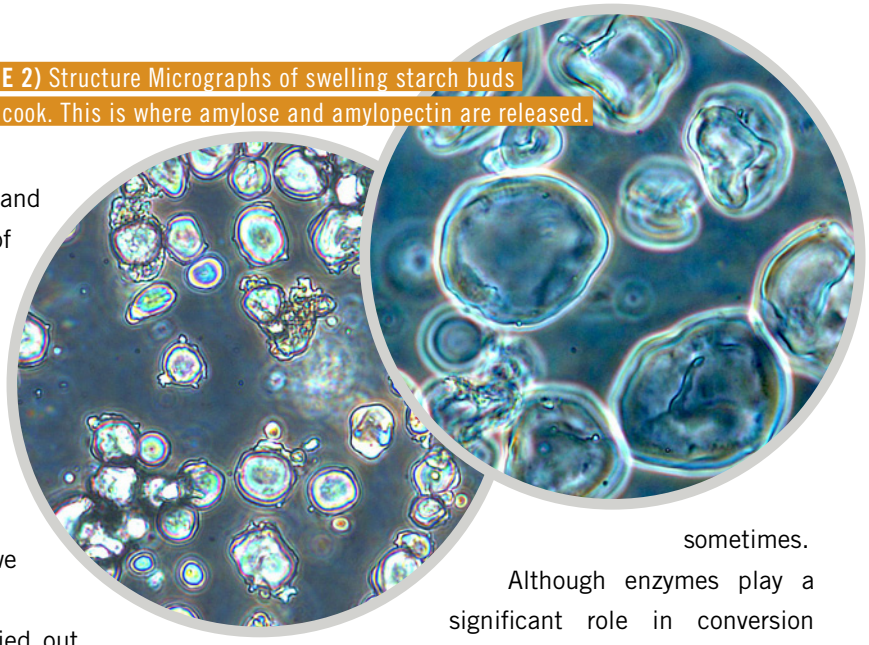
Although a substrate with readily fermentable sugars like molasses may not require heating in terms of sugar utilization, sometimes heat is implemented to lower any potential microbial contamination, which can improve the success of fermentation and ethanol yields.



(FIGURE 1) Structure of amylose (A) compared to amylopectin (B).

STARCH-BASED FEEDSTOCKS AND OTHER COMPLEX CARBOHYDRATES

Before jumping straight into fermentation of starch-based feedstocks, it is important to first discuss the relationship between fermentable sugars and more complex carbohydrates like starch. One simple analogy is that if starch is a string of beads, then each bead represents a glucose molecule (just a reminder, dextrose is the same thing as glucose and the two are used interchangeably). Starch has different conformations, and is made up of either linear chains of glucose (called amylose) or more highly branched amylopectin. Amylose uses one type of bonding to connect the glucose molecules together (alpha 1,4), whereas amylopectin uses a combination of those same alpha 1,4 bonds as well as alpha 1,6 bonds. The numbers (1,4 or 1,6, in this case) describe which carbons on each glucose are connected, so a 1,4 bond means that carbon #1 of one glucose molecule is connected to carbon #4 of the next glucose molecule in the chain. The alpha 1,6 bonds are where the branching occurs. Thus, starch is composed of fermentable sugars, they just first need to be released before the yeast can utilize them. It makes sense that the less complex the molecule (in this case amylose), the easier it is to release those glucose subunits. Thus, feedstocks where the starch has a higher amylopectin ratio (certain rice cultivars, for example) may be more difficult to convert to fermentable sugars, compared to one with a higher ratio of amylose. Figure



(FIGURE 2) Structure Micrographs of swelling starch buds during cook. This is where amylose and amylopectin are released.

1 shows the basic structure of amylose (A) versus amylopectin (B). Note the difference in structure between amylose (A; linear; unbranched) and amylopectin (B; branched). Also notice the location of the alpha 1,4 and 1,6 bonds and how the carbons are connected. Also worthy of mention is that although glucose is the basic subunit of starch, there are other configurations of glucose that are fermentable by yeast. For example, maltose (a disaccharide consisting of two glucose subunits) is fermentable, so it isn't a requirement that starch be broken all the way down to glucose to be fermentable. This is explained below as we get into malt-mediated starch conversion.

Conversion of starch into fermentable sugars is carried out by biological catalysts called enzymes. Enzymes that break down starch into fermentable sugars include alpha- and gluco-amylases, among others. These enzymes cleave the starch in different ways such as at the 1,4 or 1,6 bond, from within the molecule or from the outside, which we won't go into detail here due to the complexity and potential confusion that may result. Starch-degrading enzymes are produced when seeds like barley and other grains are germinated/sprouted. If you think about how plants get their energy from sunlight, then it makes sense that a developing seedling underground and away from light would need an alternate plan for making energy. Thus, during seed germination, enzymes are produced that convert the starch into useable (fermentable) sugars/carbohydrates (maltose and glucose, for example) that the seedling uses for generating energy until it grows above ground and can use sunlight for photosynthesis. An additional benefit of malted grains apart from producing enzymes that hydrolyze/breakdown starch is that the released sugar from enzyme activity lends a sweetness to the mash. Distilleries that ferment grain but do not incorporate malt can use commercial enzymes to convert the starch to fermentable sugars. It is also important to note that when malted grains are used as the sole source of enzymes the resulting sugars include primarily maltose, but also some glucose.

In many cases, even distilleries that use malted grains will supplement with commercial enzymes as it can significantly improve yields. In our experience with hundreds of distilleries, the primary benefits to supplementing with enzymes are yield improvement (less residual sugar and more ethanol at the end of fermentation) and reduced fermentation times. Supplementing with enzymes may also be necessary in seasons when there are issues with enzyme activity in the malt, which happens sometimes.

Although enzymes play a significant role in conversion of starch to fermentable sugars, there are other factors that are involved, namely enzyme dosing, temperature, pH and time. For example, many alpha amylase enzymes require temperatures of around 185°F for optimum activity, which is why distilleries normally cook corn at temperatures in that range. Smaller grains like wheat and rye are typically cooked at lower temps (165°F, for example) due to differences in how starch in those grains gelatinizes and other factors like protein content, etc. Malted grains are usually cooked at the lowest cook temperatures (145°F, for example) compared to other grains to prevent destroying the enzymes, and for that reason are often added last when making multi-grain mashes for bourbon or whiskey production. pH is also a factor and is dependent on the type of enzyme. Once the starch and the right enzyme dose is mixed together in solution at the right temperature and pH, conversion to fermentable sugars in not immediate. Thus, the final factor is time. In most grain-based mashes, two hours is a good place to start when considering residence time for cook relative to starch conversion.

One must also consider the amount of starch in the grain as another determinant of success since it is directly related to the amount of fermentable sugar, which determines the ethanol production potential. Corn should have between 65 and 74% starch, for reference. Milling is also important as the grains must first be broken down into fine particles to increase surface area and expose starch to the enzymes. These factors, although highly important, were de-emphasized here for the sake of time.

Figure 2 shows a microscopic view of starch buds containing amylose and amylopectin as they swell during the cook process from heat and water intake. It is during this expansion and subsequent bursting of the starch buds that the amylose and amylopectin are released, exposing the long chains of glucose

to the enzymes for breakdown.

Other complex carbohydrates like cellulose and hemicellulose, and structural components of plant biomass like corn stover or wheat stubble, have similarities to starch in that they are composed of long chains of sugars, including glucose, that once released can be fermented by yeast. However, cellulose, unlike starch, is a structural component and the bonds that link the sugars together are much harder to break and require more effort compared to starch. These are more of interest to fuel ethanol producers, so we won't go into the full details here.

OXYGEN CONSIDERATIONS

Another important aspect of sugar utilization is oxygen availability, which can determine whether the yeast will use sugar for ethanol production or if it will be used to generate energy for cell growth and reproduction. This is a highly complex line of inquiry and there is still a lot to learn, so we will stick to the basics. When oxygen is depleted, as is the case several hours into fermentation, the yeast must utilize one of the few available energy-generating pathways that do not require oxygen. One of these pathways is called glycolysis, which includes some of the first steps for breaking down glucose to create energy. Following glycolysis, the glucose molecule has been stripped of some of its energy potential that the yeast can use to survive and the resulting byproduct is ethanol, which still retains a good bit of the energy of the glucose. When there is an abundance of available oxygen (aerobic conditions, where air is actively added to a vessel) the yeast has more options for getting energy from the glucose molecule because it can utilize metabolic pathways that involve oxygen. Other metabolic pathways involving glucose where energy is created that use oxygen include Krebs Cycle (also known as the Tricarboxylic Acid Cycle or Citric Acid Cycle in case you weren't already confused enough) and the Electron Transport Chain. When these additional pathways are activated in the presence of oxygen the yeast can dismantle a glucose molecule to produce much more energy compared to when oxygen is depleted, which is great for reproduction and cell growth. However, when a glucose molecule is stripped of most of its energy, there is no high energy ethanol molecule left for the producer. This is why when we propagate yeast, we aerate the tanks, because we want to encourage cell growth and reproduction. In fermentation the tanks are not aerated, except on rare occasion, because we are interested in ethanol production rather than yeast growth. There are other factors relating to oxygen that complicate sugar utilization with respect to distillery ethanol yields (see Crabtree effect), which we will save for another time.

DIAGNOSING ISSUES RELATED TO SUGAR UTILIZATION

Now that we've covered the basics of sugar utilization, how do we identify and solve issues related to leftover/residual sugars at the end of fermentation? Identifying a sugar utilization problem is easy. If there are leftover sugars at the end of fermentation, there is a problem! Solving sugar utilization issues can be complex, but often relates in one way or another to what was discussed above. Table 2, Row 1 is an example of a high-yielding fermentation with low residual sugars and high ethanol. When we measure the components of fermentation using HPLC (High Performance Liquid Chromatography) the sugars are normally listed in terms of their Degree of Polymerization (DP), or how many glucose subunits the sugar has. In Table 2 the sugars are listed as glucose, maltose (which consists of two glucose subunits), maltotriose, often referred to as "DP3" due to having 3 glucose subunits, and finally "DP4+", which represents glucose polymers that are four glucose subunits or longer (thus the "+").

TABLE 2 Comparison of optimized fermentation of a grain-based mash with batches that have high residual sugars. Results shown are at 72 hours.

	DP4+	DP3	MALTOSE	GLUCOSE	LACTIC ACID	GLYCEROL	ACETIC ACID	ETHANOL
Normal batch with low residual sugars and high ethanol production	0.452	0.097	0.245	0.012	0.110	0.678	0.013	8.598
Batch with high fermentable sugars	0.678	0.112	1.731	0.987	0.121	0.523	0.011	6.487
Batch with high dextrins	2.134	1.842	0.432	0.034	0.111	0.432	0.012	6.265

DP4+ is sometimes referred to as "dextrins", and represents larger glucose polymers, which should get broken down over the course of fermentation. Table 2, Row 2 is an example of what a batch looks like with high residual fermentable sugars. If the leftover sugars are fermentable (glucose and maltose in this case) then we want to consider reasons why the yeast did not utilize the sugars. One possibility is bacterial contamination. When bacteria or contaminating microbes affect fermentation there will be high levels of organic acids (lactic and acetic acid) that accompany the residual fermentable sugars. This was explained in detail in the Summer 2015 issue of Artisan Spirit, so refer to that article for more information. If organic acid levels are normal, as we see in the example in Table 2, Row 1, but the fermentable sugars are high, then we can rule out microbial contamination and start looking at something like a potential nutrient deficiency or toxicity that is affecting the yeast. It could also be that we haven't allowed enough time for fermentation to take place. Monitoring yeast populations, viability, and budding can also help the producer determine if there are issues with the yeast resulting in poor uptake of sugar, which causes lower yields. Yeast populations should be well above 100 million cells per ml with at least 90 percent viability, for reference.

Another scenario is when the residual sugar is in the form of dextrins (DP4+ and DP3; Table 2, Row 3). This happens when there are issues with starch breakdown, which can be caused by several factors. Grain quality is a good place to start when troubleshooting issues with leftover dextrins. Milling and particle size also play an important role in how efficiently the starch gets broken down. Enzyme activity is probably the biggest factor and is related to the quality of the malt or the types of commercial enzymes being used. If supplementing with commercial enzymes, make sure you are using the correct dose and also the correct enzyme for the application. We often see high-heat enzymes designed for cook being used in fermentation and vice versa. Those simple mistakes can lead to major yield and production issues. Cook parameters like temperature, residence time and pH also contribute to whether starch is broken down completely.

CONCLUDING REMARKS

Although we have just barely scratched the surface with respect to the complexity of sugars and their role in fermentation, it is easy to see how important sugar utilization is to distillery yields and overall success. Hopefully this information will prove useful for understanding how different feedstocks are treated prior to and during fermentation, as well as for troubleshooting issues related to residual sugars. ASM

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