HARD SELTZER AND LOW NUTRIENT SUGAR FERMENTATION BEST PRACTICES

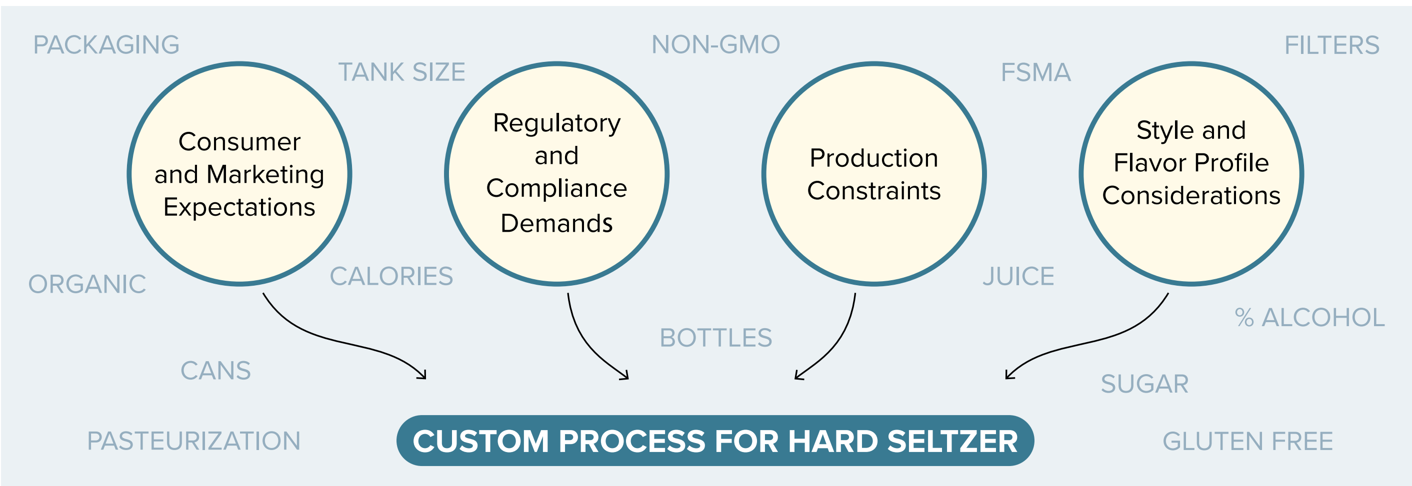
This guide is for producers who are fermenting cane sugar or other sugar substrates to produce alcoholic bases for hard seltzer and other ready-to-drink alcoholic beverages.

This guide is meant to address challenges common to low nutrient, high sugar ferments.

BACKGROUND

Hard seltzer and other ready-to-drink blends of carbonated water, alcohol, and flavoring are popular with consumers looking for low calorie, low carbohydrate, gluten-free alcoholic beverages. Several challenges need to be addressed to produce fully fermented bases in a reasonable time frame from low nutrient sugar substrates. **Without intervention, sugar fermentations are notoriously slow, unpredictable, and differ significantly from malt-, fruit-, or grape-based fermentations.**

How Will You Make Your Hard Seltzer? ***There Are Many Factors To Consider...***



NUTRIENTS ARE IMPORTANT – Yeast require a balance of vitamins, minerals, survival factors and nitrogen in addition to an energy (sugar) source to properly grow and survive during fermentation. Malt- and fruit-based ferments typically have ample amounts of nutrients, but **sugar-based ferments are nutrient deserts and supplementation is essential**. Nutrients decrease fermentation time and promote complete (dry) ferments.

NOT ALL NUTRIENTS ARE THE SAME – Nitrogen is one of the most important yeast nutrients. Yeast use assimilable nitrogen to synthesize cellular proteins, enzymes, and nucleic acids. Without adequate nitrogen, fermentation slows because yeast cannot maintain their cell function or reproduce.

We advocate using two types of nutrients for hard seltzer ferments: **Go-Ferm Protect Evolution™ used during dried yeast rehydration** and **NUTRI^{2}™ added at the start of fermentation**. GO-FERM PROTECT EVOLUTION is a cell protectant that provides natural micronutrients and survival factors directly to the yeast during rehydration. NUTRI^{2} is a complex yeast nutrient that delivers 100% natural organic nitrogen in the form of highly assimilable amino acids for growth and survival during fermentation.

Other nitrogen sources, such as diammonium phosphate (DAP) and urea, can be used to overcome some nitrogen deficiencies. However, these chemicals cannot be used in organic-labelled products and, in the case of urea, there is a high risk of ethyl carbamate formation, a compound classified by the FDA as a probable carcinogen.

Note: Nitrogen supplementation recommendations for malt, fruit, and grape fermentations are based on targeting a certain Yeast Assimilable Nitrogen (YAN) or Free Amino Nitrogen (FAN) content. However, YAN/FAN targets developed for those fermen-

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tations are often unsuccessful when applied to hard seltzer and other low nutrient sugar ferments. We have found that a more complex nutrition strategy is necessary to achieve good results and that YAN/FAN availability is only part of that strategy.

ADEQUATE YEAST DOSAGE PRODUCES BETTER RESULTS AND HIGHER GRAVITIES REQUIRE HIGHER DOSAGE RATES – High gravity seltzers are especially difficult to ferment due to increased osmotic pressure from high sugar at the start of fermentation and elevated alcohol at the end of fermentation. Even with nutrient supplementation, sugar ferments struggle when yeast dosage rates are too low.

YEAST STRAIN MATTERS – Yeast strains vary in alcohol tolerance, nutrient requirement, temperature tolerance, ability to breakdown disaccharides, fermentation speed, and many other traits. Many strains are not robust enough to ferment hard seltzer bases. Therefore, **VI-A-DRY BOSS™ yeast is recommended for its strong fermentation kinetics, low nutrient needs, neutral sensory effects, and ability to breakdown sucrose and other sugars**.

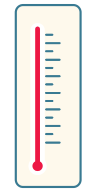
SUGAR SOURCE CAN AFFECT FERMENTATION RATE AND MONOSACCHARIDE SUGARS MAY BE FASTER TO FERMENT – Yeast are only able to utilize monosaccharide (single unit) sugars of glucose and fructose. For yeast to use disaccharides, trisaccharides, or larger sugars (sucrose, maltose, etc.) the sugar must first be broken down into monosaccharide units.

Yeast's ability to break down di- and trisaccharides into useable glucose and fructose varies and is strain dependent. Most yeast can break down sucrose into glucose and fructose by producing invertase. However, in difficult fermentation conditions, the additional yeast energy required to produce invertase may slow fermentation. Other sugar and yeast strain combinations may not be compatible and enzyme additions may be necessary to break down larger sugar molecules into glucose and fructose.

COMMON SUGAR SOURCES USED FOR HARD SELTZER FERMENTATION

- **Sucrose** (table sugar/cane sugar) – disaccharide of glucose and fructose
- **Dextrose** – glucose derived from corn
- **Invert sugar** – mixture of glucose and fructose made by sucrose hydrolysis
- **Agave** – sugar from the agave plant consisting primarily of fructose
- **Maltose** – disaccharide of two glucose molecules

IDENTIFYING OPTIMAL FERMENTATION TEMPERATURE CAN BE TRICKY – Temperature affects fermentation rate, yeast health, and sensory impact. Despite its importance, the numerous factors affecting low-nutrient sugar fermentations make it difficult to provide clear temperature guidelines. Yeast strains vary in their temperature tolerance and can be stressed when fermenting at the upper or lower end of their recommended temperature range, especially as alcohol by volume (ABV) increases. Although fermentation is faster at warmer temperatures, the risk of yeast stress leading to stuck ferments and off-odors also increases. Finding a fermentation temperature that balances fermentation speed and yeast health will likely require experimentation for any given process.



pH DURING FERMENTATION AND IMPACT ON FERMENTATION KINETICS – Sudden drops in pH during fermentation have been known to cause yeast stress and could be an issue depending on many factors (nutrition regimen, water chemistry, etc.). **In trials with our recommended products, we found decreases in pH did not negatively affect fermentation**. If you are experiencing fermentation difficulties following other protocols, pH may be an issue and using a buffer such as potassium bicarbonate may be useful.

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HOW TO USE THIS GUIDE

Given the numerous variables that exist for a producer, we strongly suggest **using the recommendations outlined in this guide as a starting point and running trials** to optimize your protocols. These guidelines and dosage recommendations are based on data from numerous lab trials, academic trials, commercial trials, and feedback from customers. We believe these recommendations provide the best chance for success given the variety of fermentation goals and production constraints that exist for hard seltzer producers.

Refer to the Hard Seltzer/Low Nutrient Fermentation Quick Guide for additional information and protocols.

<table>
<tr><th>Production Phase</th><th>Best Practice</th><th>Why?</th><th colspan="3">Recommendation</th></tr>
<tr><td>Choose Sugar Substrate</td><td>Use a monosaccharide sugar source if possible</td><td>Monosaccharide sugars (glucose and fructose) are easier to ferment and may be faster to ferment (see section above on sugar).</td><td colspan="3">Choose the best sugar for your fermentation and stylistic goals</td></tr>
<tr><td>Choose Desired ABV</td><td>Pick the lowest ABV possible for your needs</td><td>Lower alcohol seltzers are easier to ferment.</td><td colspan="3">Choose the right ABV to target for your needs. ABV may be a stylistic choice (what do your customers want?) or a practical choice (higher ABV to blend out later).</td></tr>
<tr><td rowspan="4">Calculate Fermentable Sugar Concentration for Desired ABV</td><td rowspan="3">Develop a process-specific conversion rate of sugar to alcohol.</td><td rowspan="3">Conversion rates are somewhat variable and will be specific to your process (yeast strain, fermentation conditions, etc).</td><td colspan="3">We use an average conversion rate of 17 g/L sugar = 1 % ABV when approximating alcohol potential.</td></tr>
<tr><td>ABV ≤7%</td><td>ABV 8-12%</td><td>ABV ≥ 13%</td></tr>
<tr><td>≤ 119 g/L fermentable sugar</td><td>120-220 g/L fermentable sugar</td><td>≥ 221 g/L fermentable sugar</td></tr>
<tr><td>Confirm fermentable sugar (glucose + fructose) in sugar substrate using an appropriate assay.</td><td>Some sugar substrates are mixtures of fermentable and unfermentable sugars and density measurements (Brix, gravity, Plato) or refractometer measurements measure all sugar regardless of fermentability.</td><td colspan="3">Consult supplier of sugar substrate for sugar analysis or use an appropriate assay to measure fermentable sugar (enzymatic/spectrophotometric analysis).</td></tr>
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Production Phase	Best Practice	Why?	Recommendation		
Prepare Sugar and Yeast Nutrient Slurry	Prepare sugar solution to target concentration in warm **chlorine free** water.	Chlorine can decrease yeast viability.			
			ABV ≤7%	ABV 8-12%	ABV ≥ 13%
	Add NUTRI^{2}™ yeast nutrient and vary addition based on desired ABV.	Yeast need nutrient supplementation in low nutrient ferments and need more nutrients to ferment higher ABV.	200 g/hL NUTRI^{2}	350 g/hL NUTRI^{2}	500 g/hL NUTRI^{2}
	Ensure slurry is mixed well, sugar is in solution/dissolved, and the NUTRI^{2} is not clumping.	Homogeneous solutions are easier to ferment.			
	"Boil" (>180°F) if desired	Heating solution (>180°F) facilitates sugar dissolution and slurry homogenization and may help mitigate contamination concerns. The vitamins in NUTRI^{2} may be deactivated but nitrogen and minerals are heat stable. Vitamins can be supplemented with rehydration nutrients (see below).			
	Cool sugar + nutrient slurry to < 104°F prior to yeast inoculation.	High temperatures can inactivate yeast.			
	Confirm sugar concentration and adjust as necessary.	Sugar slurries can be difficult to homogenize. Confirming sugar concentration verifies proper mixing as well as target ABV accuracy.			
Yeast Rehydration			ABV ≤7%	ABV 8-12%	ABV ≥ 13%
	Use VI-A-DRY BOSS™ yeast or another yeast that's a strong fermenter and calculate dosage rate based on potential ABV.	Yeast strains with strong fermentation kinetics have the greatest chance of success in difficult fermentations. Higher ABVs require higher yeast dosage rates.	150 g/hL VI-A-DRY BOSS yeast	250 g/hL VI-A-DRY BOSS yeast	350 g/hL VI-A-DRY BOSS yeast
	Use GO-FERM PROTECT EVOLUTION™ rehydration nutrient when rehydrating dried yeast. (1.25 x yeast dosage)	Rehydration nutrients help ensure complete and timely fermentations by supplying essential vitamins, minerals, and sterols for yeast health and protection, and will help supplement compounds inactivated during the boil (if done).	190 g/hL GO-FERM PROTECT EVOLUTION	315 g/hL GO-FERM PROTECT EVOLUTION	440 g/hL GO-FERM PROTECT EVOLUTION
	Rehydrate dried yeast according to our recommendations (see Quick Guide)	Proper rehydration ensures the viability and fermentation performance of yeast			

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Production Phase	Best Practice	Why?	Recommendation		
Inoculate and Start Fermentation	Attemperate yeast slurry prior to adding to sugar slurry (see Quick Guide)	Attemperation of the yeast slurry to within 10°C (18°F) of the sugar slurry is important because it reduces the chance of the yeast being "shocked" by the sugar slurry temp. It also helps acclimate the yeast to the sugar slurry environment.			
Fermentation	Optimize and control fermentation temperature	Optimization is necessary to identify a temperature that balances fermentation speed with yeast health. Fermentation temperature can improve fermentation rate and avoiding significant temperature changes (±10°F) in short periods of time (~8 hrs) can reduce yeast stress.	**ABV ≤7%** 27-30°C (80-85°F)	**ABV 8-12%** 27-30°C (80-85°F)	**ABV ≥ 13%** 20-25°C (68-77°F)
	Mix/stir ferments 1-2 times per day	Gentle mixing, continuously or periodically, keeps yeast in suspension, breaks up stratifications, and improves fermentation kinetics. This is especially important for larger fermentation tanks where stratification is more likely to occur.	Do not rely on natural convection/motion from fermentation to adequately mix fermentation vessels.		
	Monitor fermentation daily	Monitor fermentation to track progress of alcohol production/sugar depletion and to intervene if a stuck or sluggish fermentation occurs.	Monitor progress of fermentation by measuring density (Plato/Gravity/Brix). Do not use a refractometer to monitor fermentation.		
End of Fermentation	Measure fermentable sugar (glucose + fructose) concentration and ABV by appropriate method.	Measuring sugar and alcohol will confirm that the fermentation is complete. Density measurements are indications of fermentation progress but are not accurate for confirming the end of fermentation.	Ferment until dry (< 1 g/L fermentable sugar)		
Downstream Processing: Clean-Up, Filtration, Stabilization, Etc.	See related "**Hard Seltzer Filtration and Stabilization Best Practices**"				

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