

NABLAB NON-ALCOHOL BEER & LOW ALCOHOL BEER

A Novel Fermentation Approach to NABLAB

Non-alcohol beers and low alcohol beers (NABLAB) have been brewed throughout history for a variety of reasons including, the scarcity of raw materials, moral or religious abstinence, conformity with local laws, and personal health and well-being. Craft brewers have historically focused on stronger, higher ABV beer styles while lower alcohol styles have largely been ignored. As a result, commercial NABLAB were often dull and lacking in flavor, or presenting specific flavors that made it difficult to match the sensory profile of traditional beer styles. As demand for NABLAB grows, craft brewers are now brewing a wider variety of great tasting NABLAB styles than ever before. There are different approaches to producing NABLAB, each of which requires substantial process and recipe optimization. In this document, we provide an overview of current best practices for crafting high quality NABLAB using a limited fermentation approach.

In general, non-alcohol beers are defined as < 0.5% ABV and low alcohol beers are in the range of 0.5 - 1.5% ABV. These definitions may vary by region.

Alcohol Removal – Difficult, Costly and Diminished Flavor

A common method for producing NABLAB is to remove the ethanol from a standard beer. This can be done using either a heating and distillation approach, or through reverse osmosis.

Pros:

- › Scalable to large production volumes
- › Able to achieve 0.0% ABV (distillation)
- › Better suited for large industrial breweries

Cons:

- › Expensive equipment
- › Energy intensive
- › Significant process optimization
- › Positive flavors are stripped along with alcohol
- › Permits required for distillation
- › Higher risk of oxidation
- › Flavor matching can be difficult due to flavor losses

Limited Fermentation – Simple and Cost Effective

Lower alcohol levels can be achieved by reducing the amount of sugar consumed during fermentation. There are two main ways of limiting fermentation in this way:

1. **Arrested fermentation:** Yeast metabolism is stopped after only a small amount of wort sugar is consumed, leaving fermentable sugars remaining in the beer. This is accomplished by adding yeast to already cold wort (cold contact), rapid cooling, or pasteurization. These methods require close analytical control and can have poor flavor outcomes.
2. **Limited wort fermentability:** The quantity of fermentable sugar in the starting wort is reduced by using a modified grain bill, shortening the mash time, or mashing at high temperatures to decrease the amount of glucose and maltose, while increasing the proportion of higher molecule weight sugars. Selecting a limited fermentation yeast strain incapable of fermenting maltotriose or maltose will allow for lower attenuation. A combination of these methods can be employed to achieve lower wort fermentability. Since some simple sugars are left unfermented, pasteurization is required to stabilize the product and prevent fermentation after packaging by contaminating microorganisms.

Arrested fermentation		Limited Wort Fermentability	
Pros	Cons	Pros	Cons
Allows for use of traditional brewing equipment	Worty flavors, diacetyl, and H ₂ S are common.	Allows for use of traditional brewing equipment.	Recipe must be optimized to achieve desired flavors.
	Close analytical control required to ensure precise process timing.	Fermentation proceeds to full attenuation.	Very high mashing temperatures are required to achieve <0.5% ABV using maltotriose-negative strains.
	High risk of overattenuation.	Low risk of overattenuation	Wild maltose-negative yeast do not produce typical beer fermentation performance and flavor.
	Lack of consistency	Greater consistency batch to batch	

TABLE 1: Comparison of different methods of limited fermentation.



Achieving low ABV through low wort fermentability

Yeast strain selection

Wort fermentability is determined by the sugar profile of the wort and the ability of the yeast strain to ferment specific wort sugars. The choice of yeast strain has the most powerful effect on wort fermentability. Different yeast strains vary in their ability to ferment specific wort sugars (Table 2). The yeast strain you choose will determine the attenuation achieved in a specific brewing wort. Most brewing strains can metabolize glucose, maltose and maltotriose to achieve attenuation in the range of 75-84%. For NABLAB styles, it is important to choose a yeast strain that is unable to ferment maltose or maltotriose to achieve lower attenuation and lower ABV levels (Table 2).

Tips for reducing wort fermentability:

1. Use maltose-negative or maltotriose-negative yeast
2. Use higher amounts of special malts and target a lower starting gravity
3. Mash it at high temperature using low liquor to grist ratio





		FERMENTABILITY			
		DIASTATICUS	MOST ALE & LAGER STRAINS	MALTOTRIOSE-NEGATIVE	MALTOSE-NEGATIVE
FERMENTABILITY Higher ↓ Lower	STRAIN TYPE	Very High Attenuation	High Attenuation	Low Attenuation	Very Low Attenuation
	Glucose / Fructose 	✓	✓	✓	✓
	Maltose 	✓	✓	✓	
	Maltotriose 	✓	✓		
	Dextrins / Starch 	✓			

TABLE 2: Wort fermentability is determined by the amount and type of sugars present in the wort, and the ability of the fermenting yeast to metabolize these sugars. High temperature mashing and a modified grain bill can be used to decrease the amount of simple sugars like glucose and maltose and increase the proportion of larger sugars. Limited fermentation yeast strains that are unable to ferment maltotriose or maltose can be used to further reduce the degree of fermentation.

Maltose-negative strains, such as LalBrew® LoNa™, are ideal for non-alcohol styles since they only ferment glucose and fructose. These strains do not ferment either maltose or maltotriose; as a result, fermentation times are very short (Fig. 1). Lower mash temperatures in the range of 70 - 74°C will typically result in attenuation of 10 - 15% with maltose-negative strains. When using a low gravity wort in the range of 5.0 - 8.0°P, an alcohol level of < 0.5% ABV is easily achievable. Most maltose negative strains are non-*Saccharomyces* species that are POF+ and are not well adapted to fermenting wort and producing typical beer flavors. The LalBrew® LoNa™ strain is the first true *Saccharomyces cerevisiae* strain that is POF-negative and selected to produce a clean and neutral ale sensory profile without fermenting maltose.

Maltotriose-negative strains such as LalBrew Windsor™ and LalBrew CBC-1™ are ideal for low alcohol styles. In a typical brewing wort (OG 5.0 - 8.0°P, mash temp 64 - 68°C), these strains will typically achieve 65 - 72% attenuation and < 3.0% ABV in traditional lower ABV styles such as ordinary bitter. Much higher mash temperatures in the range of 82 - 86°C are required to produce lower ABV levels and it is difficult to achieve < 0.5% ABV using these strains. Mash temperatures above 86°C are not recommended as this promotes spontaneous hydrolysis of larger sugars into smaller fermentable sugars resulting in higher attenuation and higher ABV.

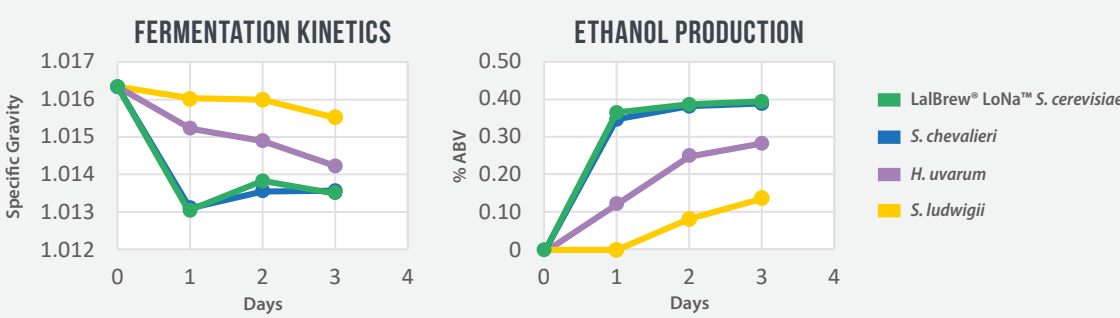


FIG. 1: Fermentation kinetics (left) and ethanol production (right) for different species of maltose negative yeast. LalBrew® LoNa™ (*Saccharomyces cerevisiae*) is compared to wild yeast strains *Saccharomyces chevalieri*, *Hanseniaspora uvarum*, and *Saccharomycodes ludwigii*.

High temperature mashing

Brewers are well familiar with the typical brewer's window of mashing temperature between 62 - 70°C. Typical mash temperatures around 62 - 64°C favor activity from β -amylase and the formation of greater amounts of maltose. As the mash temperature increases, the β -amylase activity decreases and the α -amylase activity increases (Figure 2), which promotes the formation of larger sugars and dextrins, which reduces wort fermentability (Figures 3 & 4).

The high temperature mash method is a modification of the standard single infusion mashing method. Malts are mashed at a low liquor to grist ratio and at high temperatures between 70 - 82°C. Maltose production is reduced since the β -amylase enzyme is rapidly deactivated above 65°C.¹ Starch breakdown will still occur due to α -amylase activity, which is stable for longer periods at higher temperatures. The resulting wort is enriched in unfermentable dextrins and high molecular weight (HMW) sugars with lower amounts of fermentable glucose and maltose compared to a standard mash (Figure 5). High temperature mashing can be combined with fermentation with maltose-negative or maltotriose-negative yeast to reduce fermentability to a minimum (Figure 4).

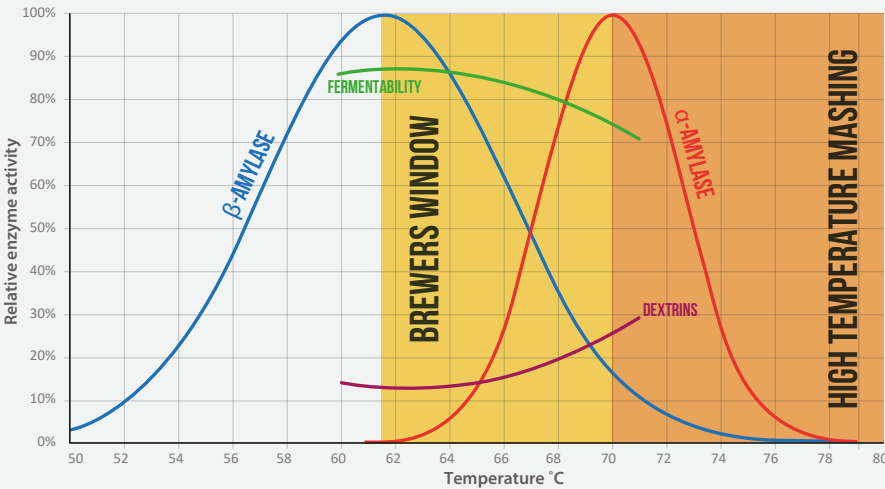


FIG. 2: Different types of enzymes have different specific activities during mash conversion that influence the composition of fermentable and non-fermentable sugars in the wort.

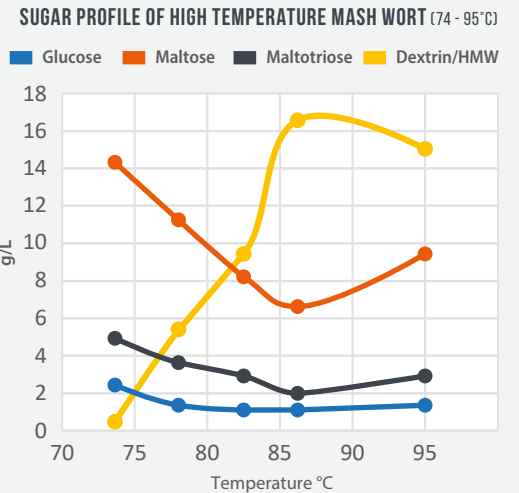


FIG. 3: The sugar profile of wort produced by high temperature mashing between 74 - 95°C. A 60 minute mash was performed at different temperatures before collecting wort. Highest concentration of dextrin/HMW sugars and lowest concentration of simple sugars were observed at 86°C. At 95°C, dextrin/HMW sugars began to hydrolyse spontaneously resulting in lower dextrin levels and higher maltose levels.

FERMENTABILITY vs MASH TEMPERATURE FOR SPECIFIC YEAST STRAINS

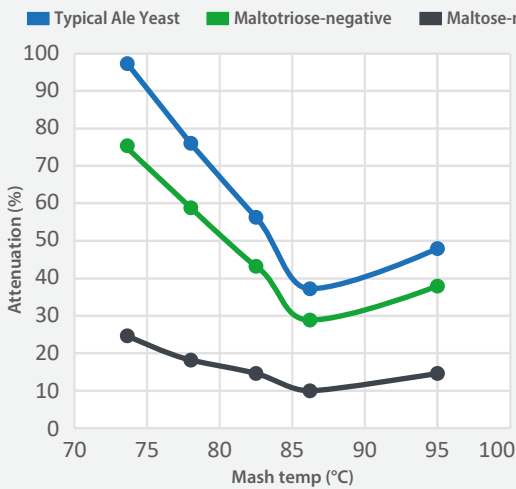


FIG. 4: The type of yeast strain has the greatest impact on wort fermentability. Higher mash temperatures further reduce fermentability by limiting the percentage of sugars that are fermentable by each type of yeast strain. A 60 minute mash was performed at different temperatures before collecting wort. Lowest wort fermentability was observed at 86°C. No sparge was done, which resulted in slightly higher attenuation than expected in a typical wort. At 95°C, dextrins began to hydrolyse spontaneously resulting in lower dextrin levels and higher maltose levels.

SUGAR PROFILE OF TYPICAL (65°C) vs HIGH TEMP MASH (70°C)

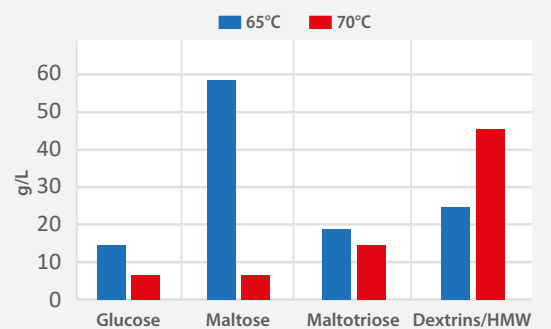


FIG. 5: A comparison of sugar profile of wort produced by a typical mash at 65°C vs a high temperature mash at 70°C. At higher mashing temperatures, the amount of unfermentable dextrin and high molecular weight (HMW) sugars is increased, while the amount of simple sugars such as glucose and maltose is decreased. Standard mash was at 65°C for 45 minutes. High temperature mash was 70°C for 45 minutes followed by a mash out at 75°C. Original gravity was 12°P in both cases.

Modified grain bill

A lower original gravity (5.0 - 8.0°P) is usually targeted for NABLAB styles, which reduces the quantity of potential fermentable sugars. A higher original gravity can be used if the beer will be blended to the intended target strength after fermentation. Higher proportions of special malts can be used, not only to further decrease the amount of fermentable sugars, but also to increase the body and mouthfeel in a low gravity wort. Some optimization may be required in order to achieve the desired sensory profile in the absence of alcohol.

Flavor control in limited fermentation

The very short fermentation time associated with limited fermentation NABLAB has implications for flavor development. A shorter fermentation window results in less yeast metabolism compared to standard beer fermentations, which is associated with:

- › Inefficient reduction of warty aldehydes
- › Reduced reabsorption of diacetyl (for arrested fermentations)
- › Decreased reabsorption of off-flavors by the yeast (H₂S, diacetyl)
- › Less CO₂ stripping of volatiles such as H₂S
- › Lower ester formation

The formation of positive flavor compounds and reduction of off-flavors requires special attention when using a limited fermentation approach to brew NABLAB.²

Worty aldehydes

It is well known that NABLAB often suffer from flavor defects often described as sweet or warty.³ These flavors originate from flavor active aldehydes which are created during the mash and boil. The most abundant are 3-methyl butanal, 2-methylbutanal and methional (Figure 6).

In standard beers, these aldehydes are reduced to their primary alcohols through the activity of yeast during fermentation. In limited fermentations for NABLAB, this reduction may not occur to the same degree since the fermentation time is so short. Various strategies have been suggested to reduce aldehydes in low alcohol beer, including treatment with PVPP, silica gel, extended boil time and CO₂ or nitrogen stripping of the wort.^{3,4} Recently, research has focused on selecting yeast strains for their ability to quickly reduce warty aldehydes. The effect appears to be both strain and compound dependent.⁵ Wild, non-*Saccharomyces* yeast strains are not adapted to fermenting beer wort and some may not efficiently reduce wort aldehydes resulting in warty off-flavors (Figure 7).

Diacetyl

During a classical beer fermentation, the yeast produces α-acetolactate, which is then excreted out of the cell. The α-acetolactate is then decarboxylated into diacetyl and reabsorbed back into the yeast at the end of fermentation where it is metabolized into acetoin, a flavorless compound. Production of α-acetolactate is increased when valine biosynthesis is more active, which occurs when amino acid levels are low as is the case for very low gravity worts. Nutrient additions to low gravity worts may reduce α-acetolactate levels and diacetyl formation. If yeast metabolism is stopped early before full attenuation, the yeast may not be able to completely reabsorb diacetyl from the beer and residual α-acetolactate may remain in the beer, which could be decarboxylated to form diacetyl in the packaged product. There is therefore a greater risk of diacetyl formation when using an arrested fermentation approach. A high temperature mash and limited fermentation yeast approach is recommended to keep diacetyl levels to a minimum. Specific limited fermentation yeast strains will also have different propensity to produce diacetyl in the finished beer (Figure 8).

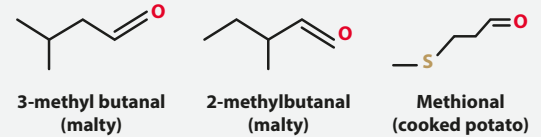


FIG. 6: Chemical structure of the most abundant wort aldehydes. Sensory descriptors are indicated in parentheses.

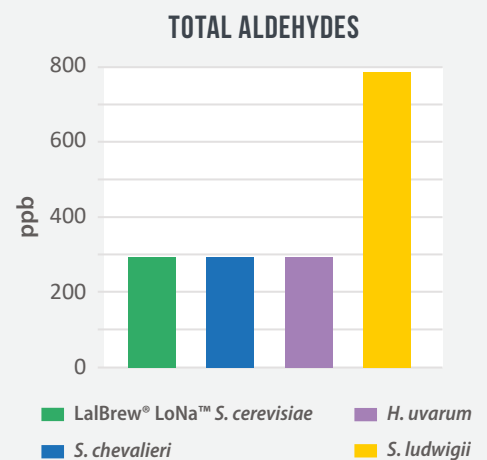


FIG. 7: Total aldehydes present in beer fermented by different species of maltose-negative yeast. LalBrew® LoNa™ (*Saccharomyces cerevisiae*) is compared to wild yeast strains *Saccharomyces chevalieri*, *Hanseniaspora uvarum*, and *Saccharomyces ludwigii*.

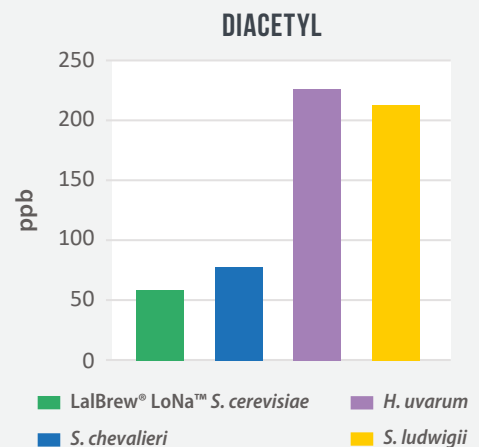


FIG. 8: Diacetyl levels of beer fermented by different species of maltose-negative yeast. LalBrew® LoNa™ (*Saccharomyces cerevisiae*) is compared to wild yeast strains *Saccharomyces chevalieri*, *Hanseniaspora uvarum*, and *Saccharomyces ludwigii*.

H₂S

Similar to diacetyl, H₂S is produced during fermentation and reabsorbed by the yeast at the end of fermentation.⁶ If yeast metabolism is stopped early before full attenuation, the yeast may not be able to completely reabsorb H₂S from the beer.

There is therefore a greater risk of H₂S formation when using an arrested fermentation approach. A high temperature mash and limited fermentation yeast approach is recommended to keep H₂S levels to a minimum.

Acidity and pH Control Guidelines

The importance of pH should not be overstated. pH is critical to the function of mash enzymes and has a major influence on hop utilization, extraction of astringent flavors from malt and microbiological stability. The pH should be maintained at acceptable levels at all stages of production, including acidification of the sparge water.

The wort pH should be adjusted pre-fermentation to 4.6 or less to inhibit growth of pathogenic bacteria. This can be done by using acidulated malt in the mash, kettle souring the wort using lactic acid bacteria (WildBrew Sour Pitch™ or WildBrew Helveticus Pitch™), or adding food grade acids.

A final pH at the end of fermentation of < 4.3 is important for product stability and may be adjusted post-fermentation using food grade acids.⁷

A final pH pre-package in the range of 3.7 - 4.1 is recommended for optimal flavor and to aid microbiological stability.⁸

In addition to pH control, acidulants can be used to modify or improve flavor. Different acids can be chosen depending on the flavor profile desired. In brewing, it is most common to use lactic acid, but citric, phosphoric, tartaric and malic acid can all be considered.⁹

NABLAB, Food Safety, and Pasteurization

Why Food Safety?

Beer is normally considered a food-safe product where pathogens are inhibited due to several factors including: alcohol content, anaerobic environment, low pH, and hop additions. These factors are reduced in NABLAB causing these beers to have greater susceptibility to microbiological spoilage, and in some circumstances, potential to support the growth of harmful pathogens. These concerns need to be addressed in a food-safe way.

Ethanol content between 3.5 - 5% by volume and a low pH (between 3.7 - 4.1) are two major factors in limiting microorganism growth in beer.¹⁰ However, some foodborne pathogens, for example *E. coli* O157:H7 and *Salmonella* Typhimurium, have been known to survive in low to mid alcohol beer (2.7 - 5.0% ABV).¹¹ In very low alcohol beer (≤ 0.5% ABV), these pathogens can be more prevalent and may grow as the pH increases from 4.0 to 4.3 and above.¹¹

Phenolics / POF

Most limited fermentation yeast strains are wild, non-*Saccharomyces* species or wild *Saccharomyces* variants that will produce 4VG (clove) and other phenolic flavors. While this may be desired in certain styles, it limits the application to more neutral beer styles. LalBrew® LoNa™ is the first *Saccharomyces cerevisiae* maltose-negative strain that is POF-negative and well suited for non-phenolic beer styles.

Esters

Ester formation is much lower in limited fermentations. Acyl-CoA and fusel alcohols are precursors of ester formation. Lower gravity wort and very short fermentation time of limited fermentations lead to less yeast growth and therefore fewer fusel alcohols. In early stages of fermentation, acyl-CoA is being used for yeast growth instead of ester formation. Esters therefore do not contribute significantly to the sensory profile of limited fermentation NABLAB.

pH Adjustment Guidelines

- ▶ **Wort/Pre-fermentation:** < 4.6 to inhibit pathogenic bacteria
- ▶ **End of fermentation:** < 4.3 for microbiological stability
- ▶ **Pre-package:** 3.9-4.1 for optimal flavor

Final Notes on Flavor

Sugars and alcohol contribute strongly to the overall body and mouthfeel of traditional beer styles. One issue with NABLAB styles is they tend to have a lower body and mouthfeel, thus contributing to a thin and watery flavor perception.

There are several recipe considerations that can help offset these flavor issues. For example, high temperature mashing and a recipe containing more raw grains and specialty malts will help increase body. Different types of acid additions may also influence mouthfeel. For NABLAB styles, it is also recommended to reduce bittering hop additions relative to comparable traditional styles to avoid harsh bitterness.

Yeast autolysates, such as AB Vickers ISY Enhance™, can be used to improve body and mouthfeel by adding flavor positive mannoproteins as well as masking harsh bitterness or astringent flavors, thus overall increasing drinkability.

3 Steps to ensure Food Safety for NABLAB

1. **Control pH throughout production**
2. **Stabilize by pasteurization**
3. **Verify microbiological stability by lab tests**

1. Controlling pH throughout production

In general, a low pH (around 4.0) limits the growth of microorganisms in beer in a couple of important ways. Within the cell, a low pH will allow more organic acids to enter, thereby increasing the intracellular acidity, which reduces the nutrient uptake and causes eventual cell starvation and death. A low pH will also enhance the antimicrobial properties of hops.

Because NABLAB contain a high level of fermentable sugars and do not have the protections of ethanol, high hop usage, and a potentially high pH, it is important to appropriately stabilize these beers. Reducing the beer pH to 4.0 offers one degree of protection; however, microorganisms can still unintentionally find a home in these beers if they are not stabilized properly.

2. Stabilization methods

PASTEURIZATION

Pasteurization is considered the most robust form of stabilization, preserving the beer in the package (tunnel pasteurization) or prior to package (flash pasteurization). Due to the lack of protections low and non alcohol beers inherently have, the typical PU, or pasteurization unit, for these beers tends to be higher than traditional ales or lagers. For example, a low-alcohol beer's typical PU ranges from 40 (minimum) to 60 (maximum), while for non-alcohol beer, these numbers are 80 - 120 respectively. To put into context, typical values for lager range from 15 - 25 and ales 20 - 35.⁸

In general, bacteria are more heat resistant than yeast; however, there is a wide survival rate range amongst species. For example, *Lactobacillus delbrueckii* are more heat tolerant than *Pediococcus* with both these species found to be more heat tolerant in alcohol free beer (0.5% ABV) than in a traditional 5% ABV beer.^{8,10}

TUNNEL PASTEURIZATION

Tunnel pasteurization is considered the gold standard of stabilization as it preserves the low or non-alcohol beer in the final package.

FLASH PASTEURIZATION

Flash pasteurization, where the beer is pasteurized before being sent to package, also offers a degree of protection; however, the risk does remain for pathogens to enter the final package.

DRAFT BEER CONCERNS

Regardless of pasteurization choice, low or non alcohol beers should not be served on draft due to the risk of entry of airborne bacteria or other undesirable microorganisms. This vulnerability does exist in traditional beer, where both gram positive and gram negative bacteria have been found in draft beer, thus highlighting the imperative for a holistic food safe program, including quality checks, package, and line cleanliness.¹¹

CHEMICAL TREATMENT

Chemical stabilization is not the preferred method for stabilizing NABLAB because these methods do not kill the contaminating microorganisms and are ineffective against some species.

Chemical stabilization treatments include: sulfur dioxide, potassium and sodium sorbate and potassium and sodium benzoate. These preservatives affect yeast and bacteria by inhibition (so not allowing for further growth), and they also may be subject to usage limits and labelling requirements depending on the region. Regardless of stabilization, all methods should be validated for effectiveness.

3. Quality control for microbiological stability

Similar microbiological checks can be conducted for low-alcohol and non-alcohol beer as traditional beer. These include selective media testing for wild yeast and bacteria (anaerobically and aerobically) as well as real time polymerase chain reaction (PCR) methods. It is important to note that different regions may require validation of the safety and stability of NABLAB.

For example, in the United States, the Food and Drug Administration (FDA) requires food processors to obtain approval from a "Process Authority" and undergo a process review. While traditional beer producers are excluded from this requirement; it is best practice for low or non alcohol beer producers to obtain a process review as part of Good Manufacturing Practices (GMPs).¹² Legally, non-alcohol beer producers (< 0.5% ABV) must comply with all aspects of 21 C.F.R. §117 including Hazard Analysis and Risk-Based Preventive Controls (HARPC) and Supply Chain Program requirements if the total volume of their production is greater than 5% of the brewery's gross revenue.¹²

It is important to check your region's food safety and beverage requirements to ensure that low and non alcohol beers are produced and consumed in a safe and enjoyable way.

Food Safety Resources

The Master Brewers Association of the Americas (MBAA) and the Brewers Association (BA) have also provided specific resources for food safety and for NABLAB production.



MBAA Food Safety Page



BA Food Safety



BA FDA Registration & FSMA Compliance Flow Chart for the Brewing Industry

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