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A comparison of selected lactic acid bacteria for use in the production of sour wort and beer.

Timothy Lozen

Bell's Brewery Inc., 8938 Krum Ave, Galesburg, MI 49053



2017
Master Brewers Conference
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Introduction

As soured beer becomes more popular within the brewing industry, many breweries might find it increasingly difficult to maintain brand consistency as production volumes increase. Therefore it becomes critical to utilize a souring organism that not only produces a good amount of favorable lactic acid in a relatively short amount of time, but also contributes other positive organoleptic properties with a minimal potential to produce off-flavors. In this study eight different LAB strains were initially selected to test for acid production as measured by pH, titratable acidity, lactic acid by reflectometry, as well as eight primary sensory characteristics.

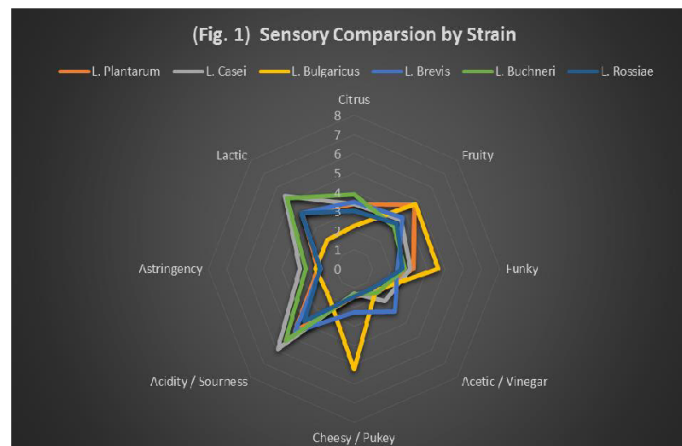
The primary division of *Lactobacillus* species is based on the metabolism of glucose. Obligately homofermentive strains produce almost entirely lactic acid from glucose. Obligately heterofermentive lactobacilli ferment to produce lactic acid, CO₂, acetic acid, and/or ethanol. A third category, known as facultatively heterofermentative, will produce CO₂ and other byproducts only under certain conditions or from specific substrates. We initially selected eight different LAB strains to test for initial growth in semi-aerobic conditions, acid production, and their sensory characteristics. The following strains were picked for their availability and reputation for use in the brewing industry for wort or beer acidification: *Lb. brevis*, *Lb. casei*, *Lb. buchneri*, *Lb. delbrueckii* ss *bulgaricus*, *Lb. delbrueckii* ss *delbrueckii*, *Lb. delbrueckii* ss *lactis*, *Lb. plantarum*, & *Lb. rossiae*.

Strain	Characteristics	Source
<i>Lb. brevis</i>	Obligately Heterofermentive	Bell's Brewery (ID w/ PCR)
<i>Lb. casei</i>	Facultatively Heterofermentative	White Labs
<i>Lb. buchneri</i>	Obligately Heterofermentive	White Labs
<i>Lb. delbrueckii</i> ss <i>bulgaricus</i>	Homofermentive	ATCC #11842
<i>Lb. delbrueckii</i> ss <i>delbrueckii</i>	Homofermentive	ATCC #9649
<i>Lb. delbrueckii</i> ss <i>lactis</i>	Homofermentive	ATCC #12315
<i>Lb. plantarum</i> (P29916)	Facultatively Heterofermentative	GoodBelly Probiotic Supplement Beverage
<i>Lb. rossiae</i>	Obligately Heterofermentive	White Labs

Method

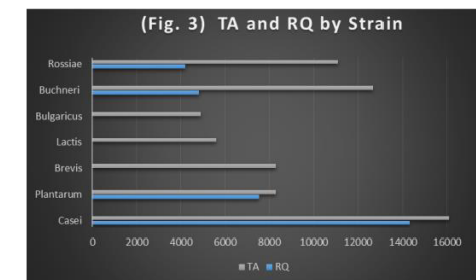
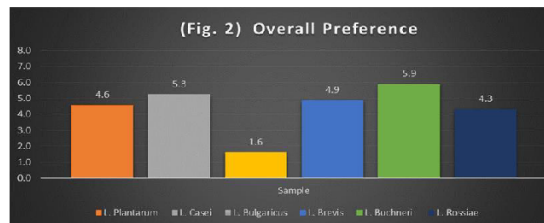
Three separate trials were conducted over the course of a month. To save on incubation space in a production environment and to minimize sensory palate fatigue when tasting acidic samples, each trial consisted of either two or three of the eight selected strains, and were grown and analyzed side by side. Each selected strain was inoculated into 9 mL of MRS Broth and incubated at 35°C for 4-5 days. Each inoculant sample was then pitched by equal volume of either 0.5 mL or 1.0 mL, depending on the age of the inoculant, into 900 mL of specialty wort for each separate trial. Each inoculated wort sample was then held at 35°C for seven days while being tested every 48 hours for Titratable Acidity (TA) by phenolphthalein indicator & pH. The specialty wort recipe was based on a traditional Berliner Weiss style and contained a blend of both pale and wheat malts while remaining unhopped. Auxiliary tests included direct lactic acid measurement by reflectometer (RQ), alcohol by volume (ABV), apparent extract, and apparent degree of fermentation by Anton Parr alcolyzer and VDKs by Gas Chromatography. On day seven a selected group of panelists, chosen for their familiarity with sour wort and beer, were blindly given two samples to judge based on eight preselected sensory metrics and also asked to provide an overall preference score. The sensory metrics were selected from data collected from a previous focus group based around soured wort sensory analysis. The average number of panelists for the sensory group was 10, with a minimum of 8 and a maximum of 13. Therefore, not all panelists participated in every trial. There was also no formal training based solely on the eight chosen sensory metrics, although panelists were selected from a pool of Bell's experienced tasters that have undergone beer sensory training.

It should be noted that two of the selected strains were excluded from portions of the experiment. *Lb. delbrueckii* ss *delbrueckii* did not produce sufficient cell density during the initial propagation phase for growth on a larger scale. *Lb. delbrueckii* ss *lactis* was not included in the sensory portion of the experiment due to abundant off flavors and aromas, presumably excluding it from use in the production of sour wort or beer.



Results

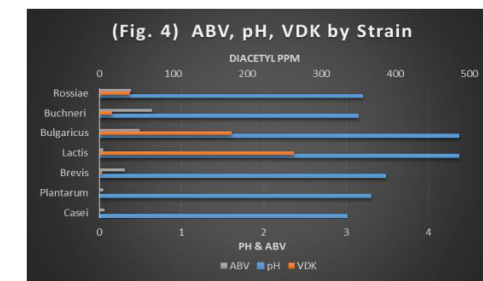
Solely in terms of overall preference score, the top three choices were *Lb. buchneri*, *Lb. casei*, & *Lb. brevis*, with *Lb. plantarum* following closely (Fig. 2). The highest scoring sensory attributes shared by these strains were acidity/sourness and lactic character, while lacking in cheesy/pukey character. *Lb. brevis* scored the highest out of all strains in acetic/vinegar character, which likely also contributed to overall acidity/sourness perception (Fig. 1). Not surprising was that *Lb. bulgaricus* scored the lowest for overall preference, while scoring the highest for cheesy/pukey character. *Lb. casei* produced the most amount of both TA and lactic acid (by RQ), 16,100 ppm and 14,300 ppm. While *Lb. buchneri* produced the highest amount of alcohol at an ABV of 0.64%, and also the second highest TA at 12,700 ppm, while only producing a modest amount of lactic acid at 4,800 ppm. *Lb. bulgaricus* and *Lb. lactis* produced the most diacetyl and also the least amount of titratable acid.



Summary

Lb. casei was the pure winner in terms of TA and lactic acid production as measured by RQ. Since we were using several heterofermentative strains, of particular interest was the difference between the RQ and TA measurement. Figure 3 shows the relationship between RQ and TA for the selected strains (RQ for *Lb. brevis* not available), and Figure 4 shows the relationship between diacetyl, wort pH, & ABV. The two facultatively heterofermentative strains, *Lb. casei* & *Lb. plantarum*, had the least amount of difference between TA and RQ as compared to the obligately heterofermentative strains, as well as the lowest ABV and VDK measurements. This suggests that the test conditions were conducive for these strains to produce mainly lactic acid, instead of ethanol, acetic acid, carbon dioxide or diacetyl. Of interest is that *Lb. buchneri* scored highly in terms of sensory preference and also had the largest difference between TA and RQ. This suggests significant production of an acid other than lactic. However, it only scored a 1.8 for acetic/vinegar character, suggesting that other acids might be pushing up the TA, possibly carbonic acid.

Further experimentation should be done to determine the ideal growth conditions and inoculation rates for individual *Lb.* strains and their effect on acidification and probably most importantly, flavor impact. Also, it is important to note that the sensory data from this study is not intended to be indicative of the contribution a particular *Lb.* strain might have in a finished product. Especially when used in conjunction with other microorganisms, such as brewers yeast.





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The Importance of Cleanliness: An Overview of CIP and COP Systems

Blake Kinnett – Sales Representative, M.G. Newell Corporation



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Sanitary equipment design is defined as the engineered design of handling, processing, storage facilities and equipment to create a sanitary processing environment in which to produce pure, uncontaminated, high-quality products consistently, reliably and economically. The universal guideline that is most useful to the food industry in this regard is Good Manufacturing Practices (21 CFR Part 110), Sec. 110.40, *Equipment and utensils*.

What is Clean-In-Place (CIP)?

CIP is a method of cleaning the interior surfaces of pipes, vessels, filters and other processing equipment without disassembly. The benefit is that the cleaning is faster, less labor intensive and more repeatable while also posing less risk to chemical exposure of workers. The cleaning cycle is comprised of different stages with water and/or cleaning solutions that require a certain time, temperature, flow, velocity and detergent concentration to achieve the necessary results. The challenge is that to remove soils, CIP solutions must reach the surface and soil to have an effect.

Typical Cleaning Sequence

Pre-flush – remove large soil/particulates
Caustic circulation – remove organic soil
Intermediate flush – flush caustic before acid cleaning
Acid circulation – remove inorganic soil
Sterilize – destroy remaining microorganisms
Final flush – flush out sterilant

Flow of liquid is responsible for carrying both hot water and cleaning solution to the soil on a surface and also for providing the physical mechanism of lifting and carrying the soil away. Studying the flow conditions during CIP can help insure the system is meeting cleaning requirements. Depending on soil load and the process layout, CIP design is typically one of the following:

- Deliver highly turbulent, high flow-rate solutions (i.e. piping and some equipment)
- Deliver solution as a low-energy spray to fully wet the surface (lightly soiled vessels with static spray ball)
- Deliver solution through a high-energy impinging spray (highly soiled or large diameter vessels with dynamic spray)

When evaluating a new CIP system (or an upgrade to your existing system), consider a few basic design requirements:

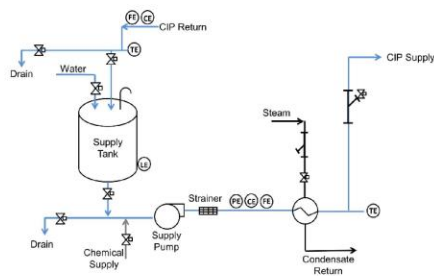
- The flow rate to clean vertical tanks with fixed spray balls is 2.5-3 gpm x tank circumference
- The flow rate to clean horizontal tanks with fixed spray balls is 0.25 gpm x surface area (sq.ft.)
- Add additional flow devices for agitators, baffles, etc.
- The effective spray distance is ~8 feet radius.
- Increase spray ball flow rate to clean outlet piping at 5 ft/second if required.
- Minimum CIP supply pressure required is 15 psi.

CIP Design Solutions

CIP systems can be designed as a single-use CIP or re-use CIP.

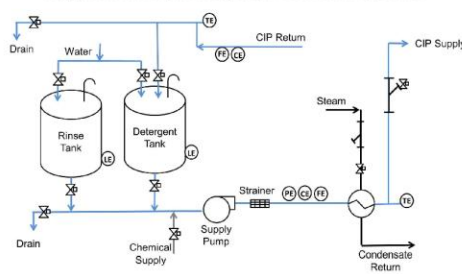
Single-Use, One-Tank CIP*

This type of system provides a lower capital investment and can be installed as a portable/skid or stationary design. Cleaning solution and water can be pumped through the system once or re-circulated.



Two-Tank, Detergent Reuse CIP*

This type of system permits reuse of wash solutions and would typically be used where water utilities are limited. System has a supply/recirculation option and typically decreased wash cycle time.



*These are typical configurations. Actual systems would be designed to meet customer requirements.

What is Clean-Out-of-Place (COP)?

COP systems are used to clean equipment parts and components. They provide consistent, repeatable cleaning with reduced chemical and water usage, less labor and faster cleaning than hand washing. COP is typically used for pump rotors, impellers, cases, hoses, tubing, fittings, gaskets and any other handling equipment.

COP washers can be designed as portable/skid or stationary systems with single or multiple compartments. Standard models are typically built with a 304L stainless steel construction with jet manifolds and a centrifugal pump to pump the water and cleaning solution through the system. Designs can be further upgraded to 316L stainless and can include a heat exchanger and PLC controls for semi-automation of sensors and feed systems.



When sizing and specifying a COP system, one should consider:

- **Industry/Application** – finish level, control and recording needed
- **Product soil type and condition** – baked on, loose, wet, etc
- **Largest component(s) that must be washed** – loading and spacing
- **Type and configuration of components** – tubing, machine parts, etc
- **Batch size** – quantity of items and turnaround time available
- **Utilities available**
- **Location in your facility** – space limitations, portable or fixed

Tips to Make Your CIP System Work for You

Tip 1 - Use vessels that are right for the process. Vessels used should be of sanitary design. Tank sanitary design includes smooth and continuous welds, self-draining and internal surfaces that are round or tubular, not flat, with no ledges or recesses. It is important that tanks are properly vented, are self-draining and that the floor of the vessel allows for fast flushing.

Tip 2 - Identify and use the right cleaning chemicals and sanitizing solutions. The chemical solution or treatment used in the CIP system must be capable of reaching all surfaces, and the surfaces are ideally made of stainless steel, not softer metals.

Tip 3 - Use the correct flow rate. For any CIP system to be effective, flow through the system must be at a high enough velocity to assure that the flow is turbulent. This means the flow must be greater than 5 ft. per second. To do this, make sure that pump sizes are sufficient for the size of the tank or length of pipes to be cleaned.

Tip 4 - Don't forget the connections. It is important that all connections in and to CIP systems are properly cleaned. In addition, avoid creating dead-ends which create difficult-to-clean sections of pipe.

Tip 5 - Monitor and verify. It is imperative that the mixing and metering of chemicals is monitored by routinely checking chemical concentrations, pH levels and monitoring pump and metering device performance. This can be accomplished by using rapid screening microbiological, chemical and environmental hygiene tests.

Tips to Make Your COP System Work for You

Tip 1 - Conduct COP tasks in order. Sanitation is a sequence of steps that build from the successful completion of the previous steps. In particular, COP practices, which involve multiple individuals working in the same area, multiple small parts to be cleaned and multiple sanitation steps for each item to be cleaned, are ineffective when steps are not taken in sequence.

Tip 2 - Consider using basket or tote washers. Another COP system that is of great value is comprised of basket or tote washers. The container is simply loaded onto the system and it passes through the unit where it is rinsed, washed and rinsed. The cleaned containers should then be stacked so that they will not become recontaminated.

Tip 3 - Use a tank rather than a rack. Parts removed for cleaning are either placed on a rack for cleaning or placed in a COP circulation tank and cleaned using a heated chemical solution and agitation. In a tank, parts may be cleaned all at once rather than individually. Also, parts in a tank can be fully submerged in the water and cleaning solution. This, along with agitation, aids in loosening soils and improves cleaning.

Tip 4 - Make sure the mechanical action tools used in COP tasks do not contribute to potential contamination. The tools used take on critical significance. Make sure that cleaning brushes are rugged, made of non-absorbent material with bristles that are resistant to retaining soils and that dry quickly. Hand brushes and floor brushes should be color-coded to ensure that those designated for use on food contact surfaces are not used on non-food contact surfaces.

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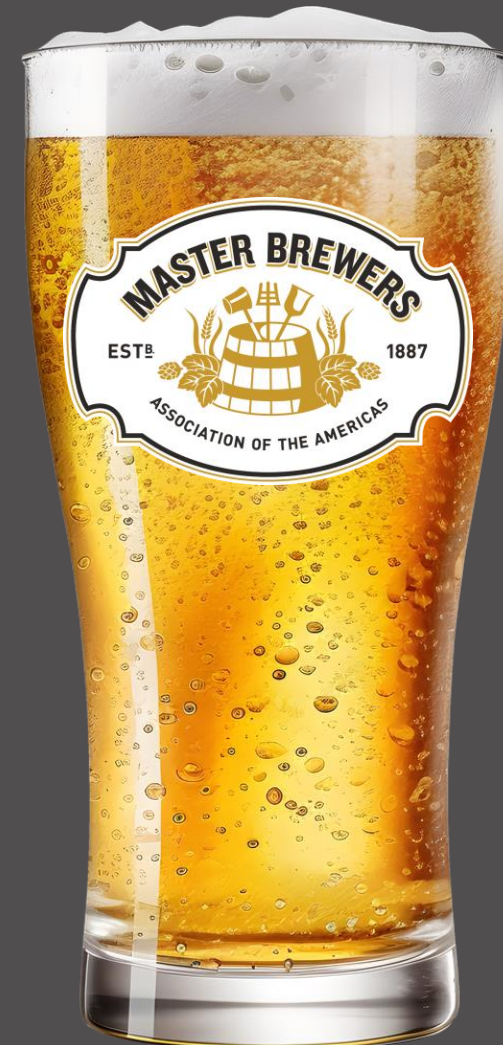
Brewing and Malting Science Course conducted by John Mallett happening Fall of 2026

Technical Quarterly

Technical Quarterly (TQ) is a leading online journal that features both peer-reviewed and non-reviewed papers covering the technical aspects of:

- Brewing ingredients
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- Beer flavor and physical stability

Have a topic or article,
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communicate next steps.





Screening of yeast strain that can efficiently utilize extracellular proline during fermentation

M-14

**Ryoya Tanahashi^{1, 2}, Glen Fox², Akira Nishimura¹, Irnayuli Sitepu²,
Kyria Boundy-Mills², and Hiroshi Takagi¹**

¹Division for Research Strategy, Institute for Research Initiatives, Nara Institute of Science and Technology, Japan

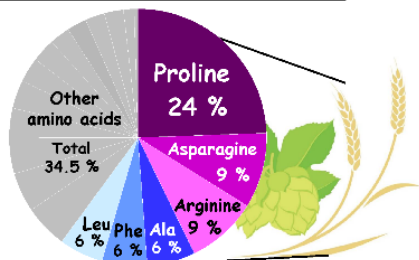
²Phaff Yeast Culture Collection, Department of Food Science and Technology, University of California Davis, USA

1. Abstract

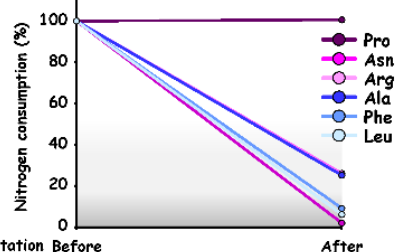
The yeast *Saccharomyces cerevisiae* plays important roles in the production of not only ethanol but also various metabolites involved in the aroma and taste of alcoholic beverages such as beer and wine. Thus, the quality of alcoholic beverages is strongly dependent on the metabolic characteristics of yeast, and nitrogen availability is one of the crucial factors in fermentation. While not a true amino acid but an imino acid, proline is the most abundant amino acid, and represents a potentially effective source of nitrogen. However, typical beer yeasts cannot assimilate proline during fermentation, leading to the high accumulation of proline in finished beer. An excess of residual proline results in risk of proline-polypheol hazes, and a bitter and mild acidic taste. The purpose of this project is to determine whether beer- or wine-associated yeasts, including both *Saccharomyces* and non-*Saccharomyces*, could utilize proline, which could improve the fermentation and final quality of beer. We first developed and validated a colorimetric assay using the dye Isatin to quantify proline in liquid growth media. Using this protocol, we found that proline was not consumed in Synthetic Complete media inoculated with an ale strain of *S. cerevisiae*, while the media inoculated with a *Lachancea thermotolerans* strain that previously known to assimilate proline had no residual proline. After this method validation, 208 additional yeasts of beer or wine origin from Phaff Yeast Culture Collection (phaffcollection.ucdavis.edu) were subjected to the screening. These included commercial brewing and wine strains as well as research isolates. Twenty-six strains consumed more than 60% of the proline from the original media, while the ale strain was unable to consume proline. To determine whether yeasts that able to consume proline could produce ethanol in wort, seven of the 26 strains, all of species *S. cerevisiae*, were used to ferment 10°Plato wort produced from dry malt extract. With Gravity and ABV were recorded each day, we found two of these strains produced 3.62 % and 2.68 % (v/v) alcohol after 10 days fermentation. These results suggest that the two proline-utilizing strains may contribute to improvements in brewing. Our next approach will include scaled-up fermentation trials to explore how these yeasts reduce to the risk of negative proline effects such as proline-polypheols hazes.

2. Introduction

Amino acids composition in wort



Amino acids consumption

Matiaž Deželak et al., *J. Inst. Brew.*, 2014

The yeast *Saccharomyces cerevisiae* plays important roles in the production of ethanol and in dictating the aroma and taste of alcoholic beverages. The quality of alcoholic beverages is thus strongly dependent on the metabolic characteristics of yeast cells. Nitrogen availability is a crucial factor in fermentation. Proline is the predominant amino acid in grape must and wort, and represents a potentially significant and effective source of nitrogen. However, yeast cells cannot utilize proline during wine and beer fermentation, leading to the high accumulation of proline in wines and beers and resulting in a bitter and low acid taste. Hence, a yeast strain that better utilizes proline would be promising for overcoming nutrient-related fermentation problems, and thereby improving the fermentation ability and quality of wine and beer.

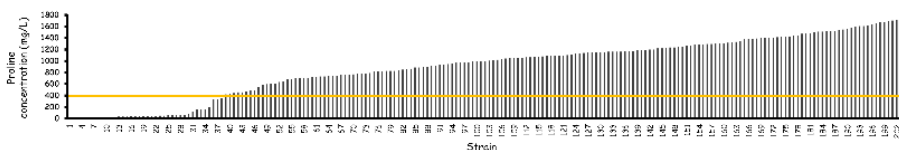
3. Development of a rapid method to measure residual proline

We first developed a rapid assay method with the dye Isatin with 96-well plate. To validate the method, residual proline was determined from cultured media with a typical ale yeast *S. cerevisiae* UCDFST 96-12, or with *Lachancea thermotorelans* UCDFST 04-833 that was previously known to assimilate proline.

5. Results of the 1st and 2nd screening

Residual proline was measured after two days growth in SC media that initially contained 1,000 mg/L proline. Strains exhibiting less than 400 mg/L of residual proline after 2 days growth were selected and subjected to the 2nd screening, in which proline consumption was assayed daily for three days.

1st screening



38 strains showed less than 400 mg/L of residual proline.

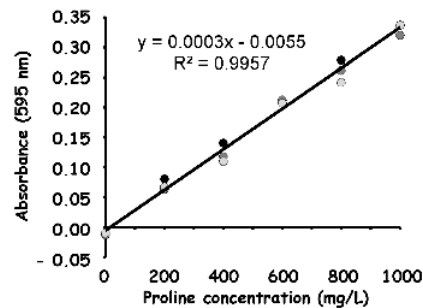
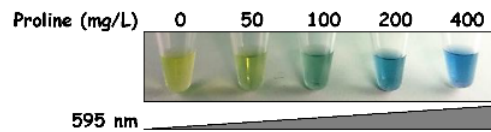
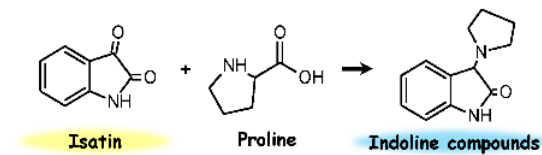
2nd screening

[illegible]

7 strains of *S. cerevisiae* (7/143; 4.9%) and 19 strains of non-*Saccharomyces* (19/65; 29.2%) consumed over 60% of the proline, suggesting that proline utilization is more common in non-*Saccharomyces* species than *Saccharomyces*.

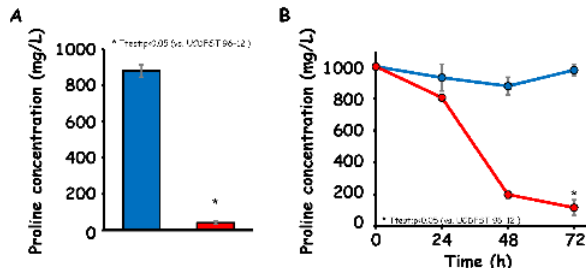
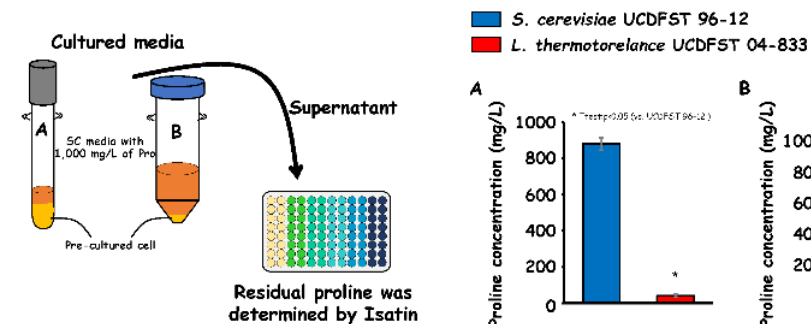
Some *S. cerevisiae* strains were able to utilize proline, indicating that genetic differences may contribute to the ability to utilize proline.

Determination of residual proline by Isatin and 96 well Plate



Isatin specifically reacts with proline and forms Indoline compounds. This reaction changes the yellow color of Isatin to blue. The standard curve showed a high degree of linearity over the working range of 0-1,000 mg/L, with a correlation coefficient of 0.9957.

Validation of the method by using media cultured with proline-utilizing strain

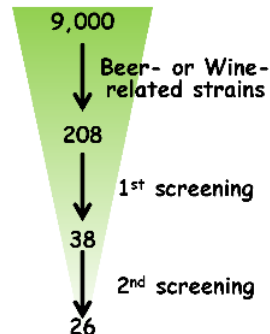


Proline remained in the media cultured with *S. cerevisiae* UCDFST 96-12 (Wyeast #1056 American Ale), while *L. thermotolerans* UCDFST 04-833 significantly consumed proline (Fig. A). The changes in proline level was also observed in the scaled-up condition (Fig. B). Taken together, we concluded that the rapid assay method was reliable in culture media.

4. Outline of screening with Phaff Yeast Culture Collection

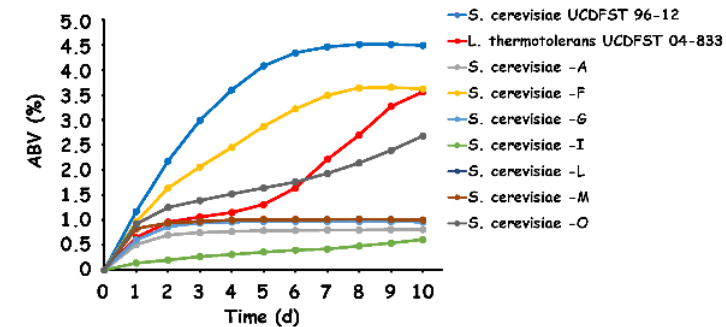


We conducted screenings with the Phaff Yeast Culture Collection, the 4th largest public wild yeast collection in the world with over 1,000 yeast species and over 9,000 strains. From the collection, 26/208 beer- or wine-related strains of *S. cerevisiae* and other species were selected from the 1st and 2nd screening.



6. Beer fermentation test with proline utilizing *S. cerevisiae*

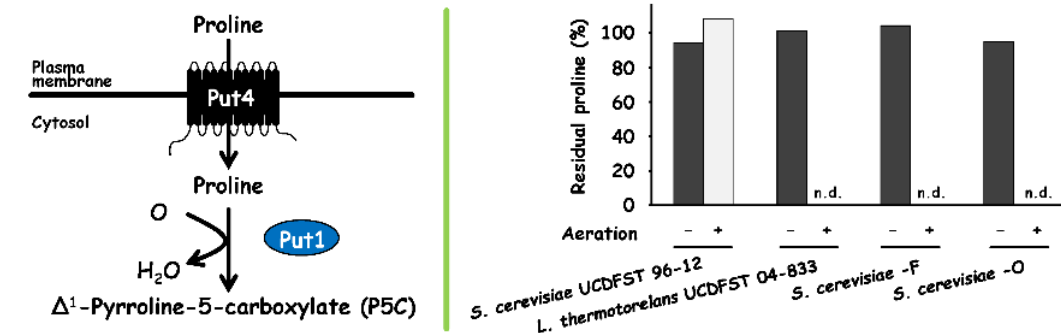
Seven strains of *S. cerevisiae* were selected from the 26 candidates. Selected strains were inoculated with wort for 10 days to determine alcohol production.



Commercial ale strain *S. cerevisiae* UCDFST 96-12 produced 4.50 % of alcohol as expected. *S. cerevisiae* -F and -O produced 3.62 % and 2.68 % alcohol after 10 days fermentation.

7. Proline consumption in wort with/without aeration

Oxygen is an essential factor to reduce proline level, since proline is degraded by the proline oxidase Put1 right after incorporated into cells. We thus tested whether strains *S. cerevisiae* -F and -O consume proline in the wort with or without aeration.



Proline was not consumed by any strains under anaerobic conditions. Under aerobic conditions, ale strain *S. cerevisiae* UCDFST 96-12 did not consume proline, but *S. cerevisiae* -F and -O as well as *L. thermotolerans* UCDFST 04-833 did. While these yeast strains were able to consume proline, the aerobic conditions required for this consumption are not compatible with brewing practices.

8. Summary

- ◆ A rapid and large-scale proline measurement method was established using Isatin and 96-well plate.
- ◆ 26 yeast strains strongly consumed extracellular proline under SC media.
- ◆ Two of *S. cerevisiae* produced low level of alcohol for 10 days fermentation and were able to utilize proline even under wort condition. Further process development is needed.

9. Acknowledgement

This study was partly supported by Project for International Collaborative Laboratories under the MEXT Program for Promoting the Enhancement of Research Universities.

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P-01

Are You a FAN of Flavor Stability?

The Amount of Free Amino Nitrogen in Your Finished Beer May Surprise You

Brewing Summit 2025

August 13–15 • Palm Desert, CA

Damase Olsson

Damaseo@hendlerfamilybrewco.com⁹

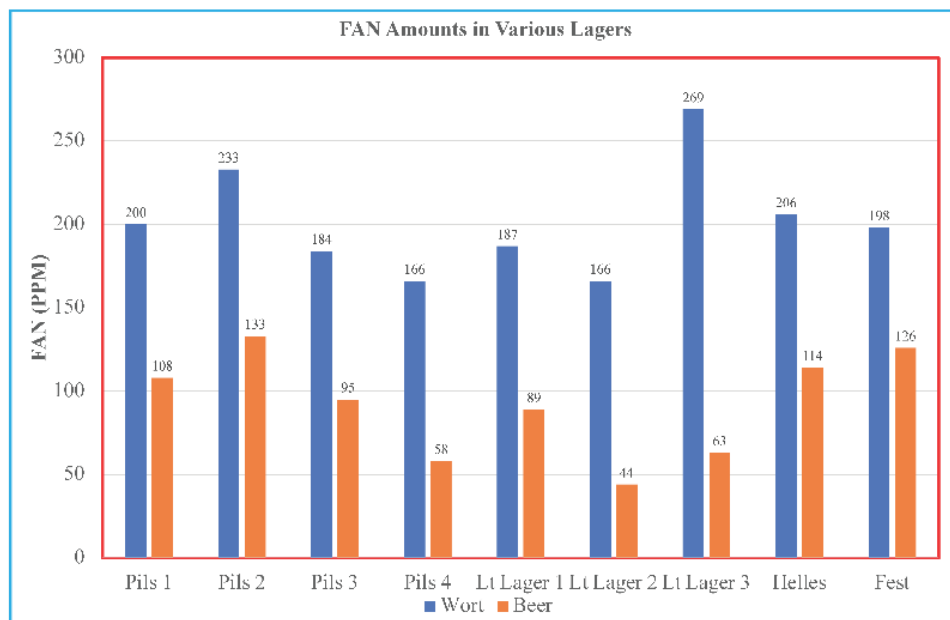
HENDLER FAMILY
Brewing Company



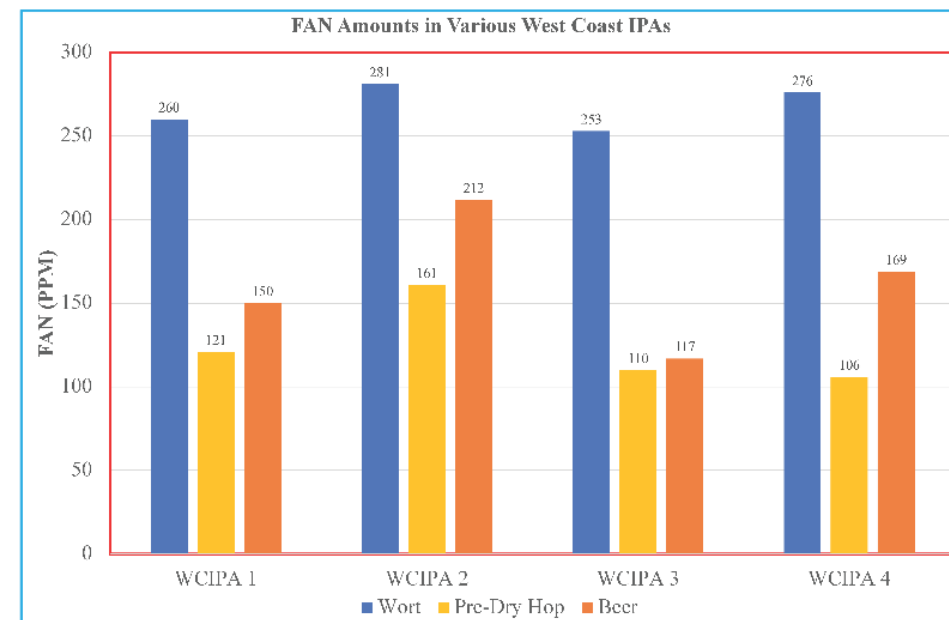
Background Information:

- Measure FAN in various styles of beer (Lagers, West Coast IPAs, and Hazy IPAs) at different points in the Brewing process, Wort, Pre-Dry Hop, and final product
- Measure the difference in FAN for two brewhouses, one with a steam-jacketed Mash tun, the other a single infusion Mash Tun with no steam-jacket
- The Goal is to see where the FAN pick up is, and where it can be possibly reduced, to aid in increasing the shelf life of the beers, as FAN can degrade to Strecker aldehydes
- Method ASBC Wort-12, the Ninhydrin test

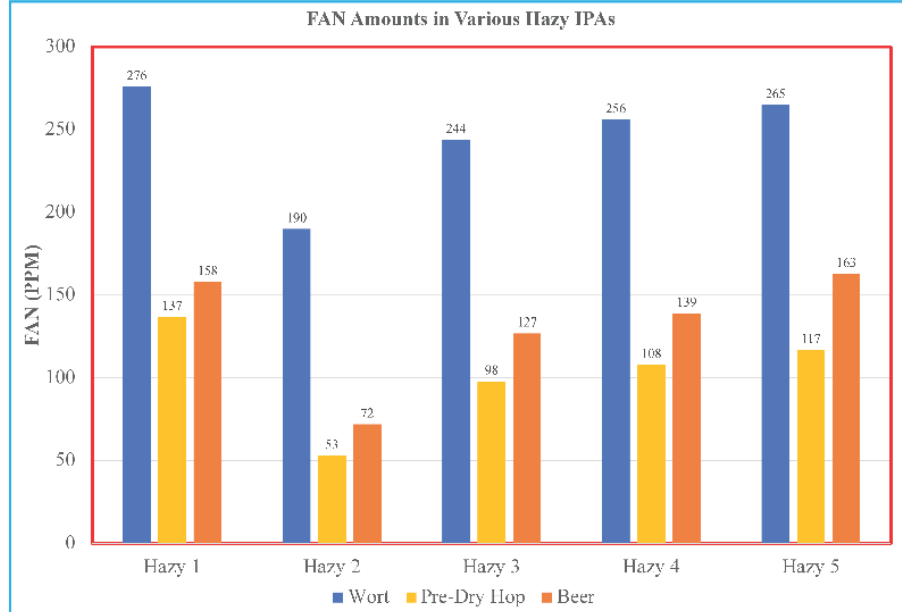
FANTastic Results:



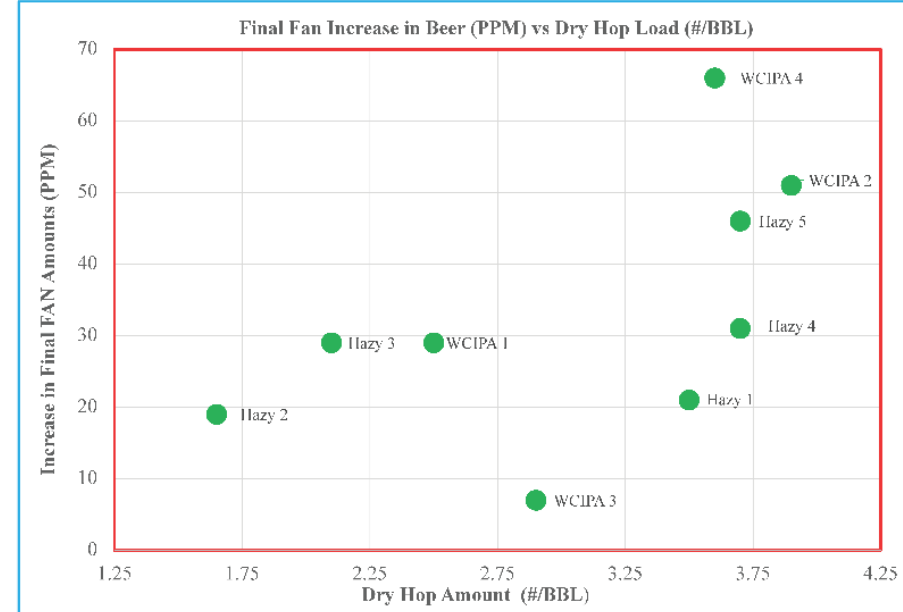
Comparison of amounts of FAN in various lagers, both in the Wort and Finished Beer (Yeast Used 34/70)



Comparison of amounts of FAN in various West Coast IPAs, in the Wort, before Dry Hopping and Finished Beer (Yeast used US-05)



Comparison of amounts of FAN in various Hazy IPAs, in the Wort, before Dry Hopping and Finished Beer (Yeast Used London Fog)



Change in the Amount of FAN from Before Dry Hopping to Finished beer, based on Dry Hop amounts

Other Interesting and FANTastic details found:

There was enough FAN in the Malt that yeast nutrients containing FAN were not needed, all the FAN values were above 14 PPM/degree Plato. We are no longer using FAN based nutrients and just minerals such as Zinc.

There was a decrease of approximately 1PPM FAN per degree Plato for the brewhouse with the “mash-out” step, as opposed to the brewhouse without that step, this was noted for 2 different beers brewed on both systems, WC3 1.6PPM/ degree Plato decrease and WC4 0.9PPM/degree Plato.

WCIPA 3 was very different comparing the Hop load to the FAN pick up when dry hopping. This beer is a beer that we dry hop using a different technique, suspending the hops in hot water (approximately 180F), and then adding them to the beer, this may cause the FAN not to increase as much as one would expect.

There was no significant change in Beer FAN while using Cryo-hops for dry hopping. But note this was done in WCIPA 3 only. We expected there to be a significant decrease in Final FAN, but since this was done on the sample with the least FAN pick up there were no changes.

We calculated yeast assimilation of FAN amounts of 34/70, US-05 and London Fog to be all comparable. 30/70 40-70%, US-05 35-60%, and London Fog 35-70% assimilation.

Next Steps and other Questions:

- Look at FAN in Wort at Kettle Full and compare between lagers and ales before they reach a boil
- Look at Whirlpool FAN before Hops are added, and compare between both ales and lagers
- Look at the effects on FAN of Tannic acid in the pulling out of proteins from the Wort
- Look at the potential Cryohops or T-45 hops for dry hopping to reduce the amount of additional FAN added during Dry Hopping in beers other than WCIPA 3
- Study why WCIPA 3 has low FAN pick up during dry hopping and can we incorporate that into some of our other dry hop regimens

A THANK YOU :

Jeremy Cross Daniel Venuto Emma Hutchins
Chris O'Connell Natalie Hicks Becky Seymour

Some FAN Humor:

What do you call FAN With a bad attitude?
Free A-Mean-O Nitrogen

What is Free Amino Nitrogen's favorite song?
Dear Mr FANTasy

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THC Beverages: Is Your Potency Staying Put?

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Introduction

Since the passage of House File 4065 in 2022, hemp-derived THC products have proliferated across Minnesota. However, minimal regulatory oversight has led to notable inconsistencies in product quality and safety—particularly in THC-infused beverages. In response, our company established rigorous quality control protocols and developed an in-house High Performance Liquid Chromatography (HPLC) testing system to monitor product potency and shelf stability. This poster presents findings from three studies investigating: (1) cannabinoid retention during long-term storage, (2) the impact of pasteurization on THC stability, and (3) the influence of flavor additives—specifically ginger—on product consistency. We tested the hypothesis that THC potency would steadily decline over time due to natural degradation. These studies underscore our commitment to delivering safe, reliable, and shelf-stable THC beverages.

Method

For all studies, THC potency was measured by injecting 5 μ L of each sample into an Agilent 1200 Series High Performance Liquid Chromatography (HPLC) using a Low Dose Cannabinoid Curve for quantification.

Study 1: Long-Term Storage Stability

Surly cans (Mixed Berry, Lime, and Passion Fruit Orange Guava (POG)) were collected from long-term storage maintained at 20–22 °C, with storage durations ranging from 1 to 2.5 years. THC potency was measured and compared to initial post-canning and Certificate of Analysis (COA) data.

Study 2: Thermal Pasteurization

Surly cans (Mixed Berry, Go Easy, and Go Forth) were pasteurized using a Jenrey tunnel pasteurizer. The process ran for 15 minutes, targeting a pasteurization unit (PU) >60, with a maximum temperature reaching 71.01 °C. Unpasteurized cans with matching timepoints were set aside. The next day, both sets of cans were tested on HPLC.

Study 3: Ginger Flavor Impact

To simulate a tank, four 1-gallon batches of de-aerated water (DAW) were dosed with 10.8 mg THC per 12 oz. serving. In three of the batches, 3 mL of ginger flavors were added. A control sample was prepared without the addition of ginger flavoring. Samples were tested on HPLC once a week for three weeks.

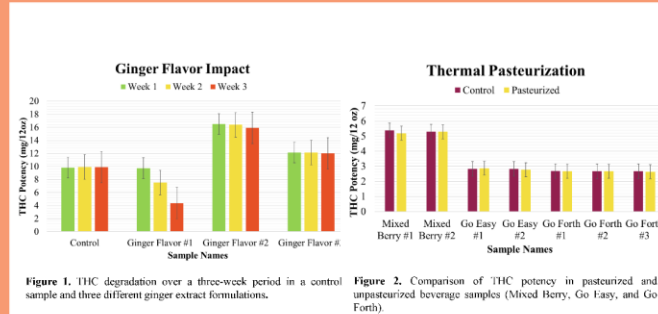


Figure 1. THC degradation over a three-week period in a control sample and three different ginger extract formulations.

Figure 2. Comparison of THC potency in pasteurized and unpasteurized beverage samples (Mixed Berry, Go Easy, and Go Forth).

Long-Term Storage Stability Mixed Berry

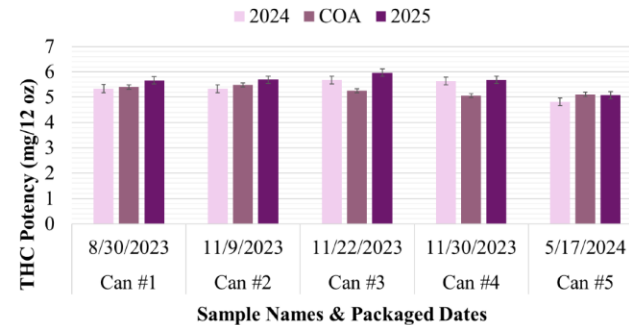


Figure 3. THC potency results over time of Mixed Berry cans from 2023, 2024, and 2025.

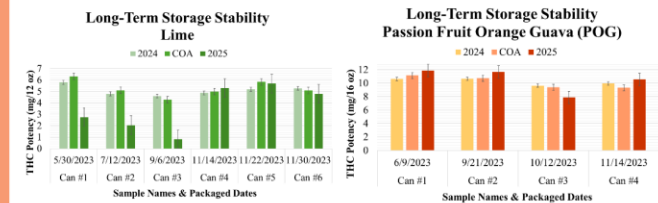


Figure 4. THC potency results over time of Lime cans from 2023, 2024, and 2025.

Figure 5. THC potency results over time of Passion Fruit Orange Guava (POG) cans from 2023, 2024, and 2025.

Result

Study 1: Long-Term Storage Stability

We assessed the THC potency stability of three different Surly THC drink flavors over an extended storage period. The results demonstrated no significant degradation of THC potency over time, indicating strong long-term stability across all three flavors.

Study 2: Thermal Pasteurization Impact

Given that THC beverages lack natural preservatives like alcohol or hops, pasteurization plays a critical role in ensuring microbial safety. To evaluate any potential impact on potency, pasteurized cans were compared to unpasteurized controls. The results showed no meaningful loss in THC potency post-pasteurization, suggesting the process is safe for maintaining cannabinoid integrity.

Study 3: Ginger Flavor Additive Effect

With the rise of more complex and unique THC beverage formulations, this study examined how flavor additives may affect potency. A bench-top study was conducted using three different ginger extracts to determine their influence on THC degradation. Notably, one of the three extracts resulted in a measurable decrease in THC potency over a three-week period, highlighting the need to carefully evaluate certain additives.

Discussion

The Surly QC Lab's objective for this project was to evaluate the long-term stability of THC beverages. Specifically, we aimed to understand how THC potency holds up over several years in our own products. Our study focused on three areas: room-temperature storage stability, the effects of pasteurization, and the impact of ginger extract on THC potency.

We initially hypothesized that THC potency would exhibit a gradual decline over time. However, contrary to our hypothesis, potency remained largely consistent, with only a few isolated cases showing minor degradation. Upon reviewing the data, we noted that recent in-house HPLC results showed slightly higher potency compared to the original Certificates of Analysis (COAs) and previous internal tests. These discrepancies may be attributed to several factors, including updated HPLC testing methods, the use of two third-party labs, and testing from different can samples. Despite these variables, all results remained within a reasonable range of the original COAs. In fact, our most recent in-house tests are within +/-1% of our third-party labs' HPLC data, which supports the validity of our current testing methods.

Future research should investigate the impact of oxygen ingress on THC stability in packaged beverages, as well as how different ingredients—such as botanical extracts, oils, and other additives—may influence potency over time.





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