Primary Journal Article Review

"Investigation of Non-*Saccharomyces* yeast strains for their suitability for the production of non-alcoholic beers with novel flavor profiles"

James Edward Brewer II

EDTL:6767: Systems Thinking in Bio and STEM Educ

Module 8

Darrin Ellsworth

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Table of Contents

Cover Letter

I selected an article outside the curated set, primarily because it offered a scientific model for a subject I am personally and professionally interested in - no-alcohol beer production. Since alcohol consumption is legally forbidden in the United States for anyone under the age of 21, it is arguably a topic unsuitable for K-12 or even university classrooms. Accordingly, I targeted my paper toward professionals in brewing and fermentation sciences and geared my presentation style toward them.

I did perform some minor edits toward making my paper more concise and readable, and eliminated some formatting errors (to conform to APA7 standards). Beyond those mechanical tasks, my aim was to impart certain learnings from our course, in an effort to make a complex topic more understandable.

To do so, I relied upon the work of Dr. Matthew E. Lira and Dr. Stephanie M. Gardner, who in their analysis of learning science frameworks, pose the question: does the presentation of new knowledge cause the individual to merely retrieve knowledge, or does it challenge them into a process of constructing knowledge? My objective was to encourage my audience to think dynamically and strive toward the latter, thus fostering "productive thinking and disciplinary engagement" (Lira & Gardner, 2020). I see this as particularly important for the professional audience I am targeting, mainly because of the increased demand for flavorful beers that contain little alcohol (< 0.5% alcohol by volume).

The Structure/Function/Mechanism model is an excellent means of describing production of non-alcoholic beer primarily through utilizing specific strains of yeast. The structure of the fermentation process is described early in my paper, starting with the structure of the yeast cell, and pointing out its functions (i.e., glycolysis, permease transport, pyruvate, etc.). The mechanism for ethanol creation is described only at a high level, in terms that brewing professionals might understand, and so overlooking the enzymatic behavior and genetic composition of the yeast cell itself, and particularly those

differences between the non-*Saccharomyces* that were selected for study, and the more standard *Saccharomyces* strains that produce higher-alcohol beers.

In my strategy to convey the findings of the article to the target audience, it's clear that the concepts involve a complex system that few experts in the brewing field have examined at this level of detail. I found that the work of Jacobson & Wilensky (2006) to be helpful in highlighting both the difficulties in absorbing complex systems knowledge, as well as by articulating the "learnability issue" and how much of a paradigm shift is needed to teach about complex systems such as fermentation, especially when there are many variables in yeast morphology and function that impact it. Accordingly, I tried to frame the mechanism of fermentation in terms of emergence; that is, how "local interactions of elements in a complex system at a microlevel can contribute to higher order macrolevel patterns that may have qualitatively different characteristics than the individual elements at the microlevel" (Jacobson & Wilensky, 2006, p. 16).

By describing the key experiments performed, along with the outcomes, I attempted to illustrate the experimental outcomes of utilizing non-*Saccharomyces* strains based on their inability to metabolize maltose and maltotriose (sugars than comprise most of wort), the overall low level of ethanol production from other sugars (compared to standard brewing yeasts), and the generation of a variety of pleasant flavors (while minimizing unpleasant ones). I believe this resulted in a convincing and coherent presentation of a complex technical topic.

Context

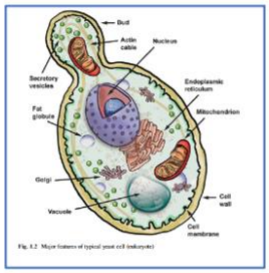
In the article "Investigation of Non-*Saccharomyces* yeast strains for their suitability for the production of non-alcoholic beers with novel flavor profiles" (Methner, et al., 2022) it is noted that "Non-alcoholic beers have seen steady growth in recent years and are attracting customer interest. At the same time, consumer demand for non-alcoholic beers is rising." (Methner, et al., p. 341). The brewing industry is responding by developing repeatable processes to economically produce "non-alcoholic" beers (> 0.5% alcohol by volume), including de-alcoholization, limited fermentation, dilution, and fermentation-free processes (Sohrabvandi, et al., 2010). The use of yeast strains from the non-*Saccharomyces* species, has not been widely attempted. This article provides evidence for utilizing such strains to produce beer low in alcohol, yet both flavorful and without sensory flaws.

Structure and Function of Yeast

The morphology of a yeast cell is shown below. The structure at this level of detail is very similar across all strains of yeast used in brewing:

Figure 1

The structure of a typical yeast cell (note the cell membrane and cell wall)



From Stewart (2017, p. 114)

Fermentation is a specialized process of a metabolic pathway called glycolysis, but unique in that it produces ethanol. This article discusses the steps to control this pathway, and thus limit the amount of ethanol generated. The diagram below illustrates the glycolysis pathways.

Figure 2

Glycolysis in the yeast cell (note the cell wall and cell membrane). The key difference between non-Saccharomyces strains and more typical brewing strains is the nature of the facilitated diffusion of maltose and maltotriose (at the upper left and right). Non-Saccharomyces strains inhibit penetration of maltose and maltotriose through the cell wall and into the cell membrane.

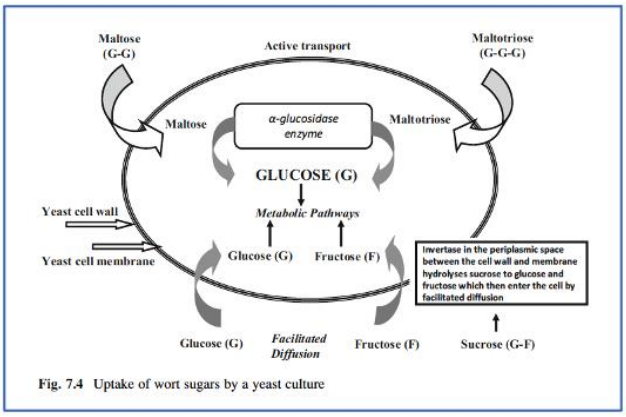


Fig. 7.4 Uptake of wort sugars by a yeast culture

From Stewart (2017, p. 114)

Teaching Approach

I will explain this article using language aligning to the knowledge of brewing professionals. I will focus on the key structural aspects of the article - its research questions, its procedures, and its outcomes – to illustrate the unique mechanisms. Also, I will cite from several technical brewing texts, including Fix (1999), Banforth (2002), and White (2010). This will add credibility by anchoring it in the wisdom of these recognized experts in the field. The objective of the audience recognizing emergence, based upon the differences in both structure and function of the non-Saccharomyces strains, and the resulting mechanisms that result in non-alcoholic beer, is supported by the research objectives of the article, and the demonstration of their success.

Article Presentation

More than 500 species of yeast exist, and thousands of different yeast strains (White, p. 17). For making non-alcoholic beer, most species are unsuitable. The reason is that wort (unfermented beer) is comprised largely of complex sugars such as maltose and maltrotriose. Non-*Saccharomyces* yeast strains cannot metabolize complex sugars, and thus cannot produce as much ethanol. Moreover, these strains cannot efficiently metabolize simple sugars, either – thus producing even less ethanol. The figure below depicts the sugar composition in the wort used for testing:

Figure 3

Sugar composition of wort used for fermentation trials (typical for industry practices). Note the proportion of maltose and maltotriose is over 85% of the total sugar composition. This fact, coupled with the hypothesis that non-Saccharomyces strains cannot metabolize these complex sugars, motivates the research questions that follow.

Sugar composition of wort in g/L	
Fructose	0.6
Glucose	4.4
Sucrose	1.6
Maltose	31.7
Maltotriose	7.3

From Methner, et al., 2022, p. 346.

Research question 1: the selected yeast strains cannot effectively metabolize maltose or maltotriose.

To test this hypothesis, a sugar utilization test was performed on each of the 16 strains. Two synthetic media were prepared - one was infused with maltose and the other with maltotriose. High Performance Liquid Chromatography (HPLC) showed the yeast strains metabolized less than 5% of the available maltose or maltotriose, unlike typical strains of brewing yeasts, which metabolize greater than 80% (Fix, p. 17).

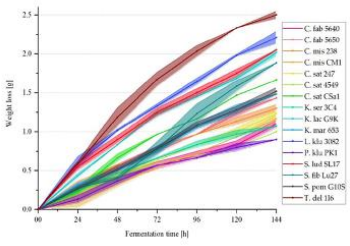
Explanation: The 16 strains were largely incapable of metabolizing maltose or maltotriose to produce ethanol. The implication is that beer produced from these strains of yeast will contain less ethanol than beer produced from more typical strains (i.e., saccharomyces cerevisiae).

Research question 2: Fermenting wort using the selected yeast strains would result in low-alcohol beer (< 0.5% ABV).

This experiment began with the same 16 strains of yeast. Wort was produced through heating water and malted barley to attain a constant sugar content. The wort was cooled and distributed into 48 glass vessels. Each strain of yeast was introduced into three vessels (16 x 3), which were sealed using glass airlocks (to facilitate release of CO_2 generated during fermentation). These were stored 144 hours at a temperature of 20° C. Fermentation progress was checked every 24 hours by noting the net weight of each vessel. Balling's method predicts weight loss due to yeast metabolizing sugars into ethanol (which has ~70% lower specific gravity than wort), and expelling CO_2 (Nohel, 2020). The graph below shows the loss from each sample.

Figure 4

Mean values with standard deviations of the fermentation sample's total weight loss during the fermentation process over 144 hours at 20 degrees Celsius. Note that an alcohol content < 0.5% ABV was observed for all but two strains. This demonstrates that neither complex nor simple sugars metabolized well.

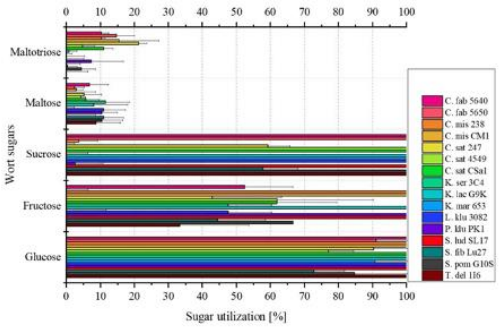


From Methner, et al., 2022, p. 345.

To confirm the observation above, another test was run to evaluate sugar utilization. These data are presented below.

Figure 5

Mean values with standard deviations of wort sugar utilization of the 16 investigated yeast strains during fermentation. Note that, on average, only about 70% glucose, 60% sucrose, and 40% fructose were metabolized.



From Methner, et al., 2022, p. 347.

Explanation: Simple sugars are indeed metabolized, but at a lower rate than with typical brewing yeasts. This is clearly shown for each strain by noting the ethanol content of the samples in the table below.

Figure 6

Mean values and standard deviations of original wort sugar content (°P), apparent attenuation, ethanol content, and pH value in beers fermented by the 16 selected yeast strains. Note that the apparent attenuation (yeast utilization) reflects ethanol content, conforming to professional brewing experience (Fix, pp. 81-83). Two beers exceeded the <0.5 ABV standard (in blue).

Yeast strain abbreviation	Original wort (°P)	Apparent attenuation (%)	Ethanol content (% *v/v*)	pH value
C. fab 5640[a]	7.05 ± 0.00	9.07 ± 0.21	0.33 ± 0.00	4.83 ± 0.00
C. fab 5650[a]	7.02 ± 0.01	12.00 ± 0.00	0.43 ± 0.00	4.79 ± 0.01
C. mis 238[a]	7.03 ± 0.00	8.93 ± 0.12	0.32 ± 0.01	4.55 ± 0.00
C. mis CM1[a]	7.03 ± 0.02	8.60 ± 0.08	0.31 ± 0.00	4.62 ± 0.00
C. sat 247[a]	7.02 ± 0.01	10.00 ± 0.00	0.36 ± 0.00	4.76 ± 0.01
C. sat 4549[a]	7.03 ± 0.00	7.73 ± 0.05	0.28 ± 0.00	4.84 ± 0.00
C. sat CSa1[a]	7.03 ± 0.01	12.77 ± 0.05	0.46 ± 0.00	4.83 ± 0.00
K. ser 3C4[b]	6.94 ± 0.01	9.23 ± 0.09	0.33 ± 0.00	4.88 ± 0.01
K. lac G9K[b]	6.85 ± 0.09	13.97 ± 0.09	0.49 ± 0.00	5.08 ± 0.01
K. mar 653[b]	6.88 ± 0.00	10.53 ± 0.12	0.37 ± 0.00	4.60 ± 0.00
L. klu 3082[b]	6.92 ± 0.00	15.73 ± 0.05	0.55 ± 0.00	4.69 ± 0.00
P. klu PK1[a]	7.02 ± 0.01	7.33 ± 0.17	0.26 ± 0.01	4.82 ± 0.00
S. lud SL17[b]	6.95 ± 0.05	14.02 ± 0.10	0.50 ± 0.00	4.92 ± 0.04
S. fib Lu27[b]	6.91 ± 0.01	13.20 ± 0.29	0.47 ± 0.01	4.60 ± 0.01
S. pom G10S[b]	6.90 ± 0.00	11.30 ± 0.16	0.40 ± 0.00	4.77 ± 0.00
T. del 116[b]	6.89 ± 0.00	15.50 ± 0.00	0.54 ± 0.00	4.78 ± 0.01

[a]Wort batch 1; [b] Wort batch 2

From Methner, et al., 2022, p. 346.

Research question 3: The resulting beer would receive positive sensory evaluations

A desirable characteristic of non-alcoholic beer is the presence of fruity flavors and aromas (Blanco et al., 2014). To assess sensory properties, ten certified assessors sampled test beers using the MEBAK sensory analysis panel (Jacob, 2013). A beer was deemed "fruity" if at least nine assessors identified it as such.

Figure 7

Number of sensory assessors identifying the beers produced with the 16 non-Saccharomyces yeast strains as "fruity". As outlined in red, all but three beer samples met this criterion.

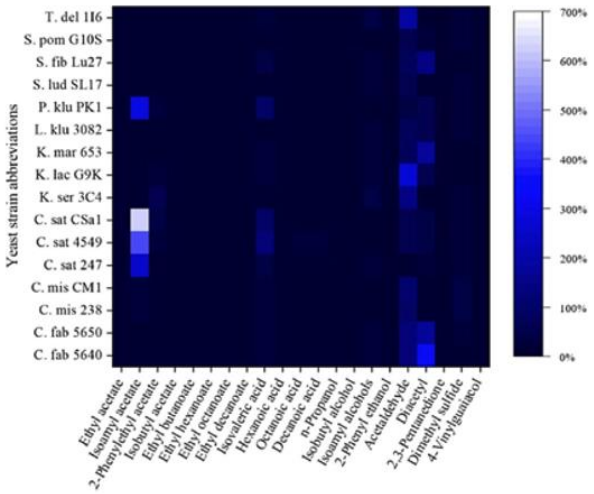
Yeast strain abbreviation	Number of assessors identifying the beer as "fruity"
C. fab 5640	10*
C. fab 5650	10*
C. mis 238	10*
C. mis CM1	9*
C. sat 247	10*
C. sat 4549	9*
C. sat CSa1	10*
K. ser 3C4	8
K. lac G9K	9*
K. mar 653	9*
L. klu 3082	9*
P. klu PK1	8
S. lud SL17	8
S. hb LuZ7	9*
S. pom G10S	9*
T. del T16	7

*Significant fruitiness (α = 0.05) according to MEBAK sensory analysis 3.1.1. and DIN EN ISO 5495:2007

Another sensory test involved the presence of by-products (volatiles) including esters, diacetyl, organic acids, higher alcohols, ketones, acetaldehyde, and dimethyl sulfide (DMS). Since volatiles are regarded as flaws in the brewing process (Bamforth, pp. 132-136), it is an important assessment in terms of commercial potential for beers from these strains, so much so that threshold levels of volatiles are accepted throughout the industry (Sannino, et al., pp 361-388). It is significant that so few of the samples actually exceed the thresholds. These results are illustrated in the heat map below.

Figure 8

Heat map of volatile compounds in final non-alcoholic beers produced by 16 yeast strains during fermentation for 144 hours at 20°C. as detected by headspace gas chromatography. The brighter the fields in the heat map, the more the threshold was exceeded. The 100% level is the threshold for volatile compounds in beer (Sannino, et al., pp. 361-388).



From Methner, et al., 2022, p. 349.

To focus on the more significant volatiles, beer flavor is particularly impacted by volatiles like acetaldehyde, diacetyl, and isovaleric acid.

- Acetaldehyde is known to cause “worty off-flavors” in non-alcoholic beers. However, acetaldehyde was noted to be accompanied by a fruity, green apple, grassy flavor in this study; therefore, it did not lead to a totally undesirable flavor impression. Acetaldehyde was detected above the threshold in six beers.
- Diacetyl has long held by professionals as a “heinous substance” with a highly undesirable “buttery” flavor and “slippery” mouthfeel at levels above 0.10 to 0.15 mg/L (Banforth, p. 119). Diacetyl was above the threshold of 0.15 mg/L in four beers.
- Isovaleric acid produces “sweaty-cheesy” to rancid flavors when exceeding the threshold of 1.5 mg/L. (Gernat, et al., 2009). Isovaleric acid was above the threshold in one beer.

Explanation: The objective of producing non-alcoholic beers with desirable flavor characteristics (yet without significant off-flavors) was achieved, on the basis of both sensory testing as well as through chromatographic analysis (Methner, et al., p. 349). An unexpected outcome was that numerous pleasant aromas were also detected during sensory evaluation, including cool mint, pear, and red berries (Methner, et al., p. 352).

Conclusion

This study demonstrated that the use of non-*Saccharomyces* yeast strains for developing non-alcoholic, flavorful beer with few flaws was successful. The practical consequence of this article, for brewmasters and fermentation scientists, is that these strains provide a practical and economic input for brewing non-alcoholic beer. An interesting follow-on question is whether similar results could obtain when scaling up to full production levels, sufficient to realize economic benefits.

This paper attempted to demonstrate that very small differences in morphology and function of yeast created a substantial difference in the outcome. Recognizing the property of emergence – a large change in the performance of the complex system of fermentation – was motivated by the intentional selection of three of the many research questions that the article addressed. I selected them with the knowledge that professional brewers would both understand and react to the outcomes, and be able to "connect the dots" from simple components to complex outcomes (Jacobson & Wilensky, 2006)

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