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Production of Esters by Saccharomyces cervisiae Fermentation of Different Fusel Alcohols

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WSU Student Research & Creative Projects
Abstract / Executive Summary / Final Report

Project Title: Production of esters by Saccharomyces cervisiae fermentation of different fusel alcohols

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Faculty Sponsor Name: Dr. Mann

Department: Chemistry

Abstract (paste below or attach):

attached

The end product of this research or creative project in electronic format has been submitted to the Provost / VP for Academic Affairs via the Office of Grants & Sponsored Projects, Maxwell 155, bayers@winona.edu.

Student Signature: Danielle Sherman Date: 11/3/14

Faculty Sponsor Signature: Jessica Mann Date: 11/04/14

Danielle Sherman

Chemistry Seminar (Friday)

Abstract and Title

Production of esters by *Saccharomyces cervisiae* fermentation of different fusel alcohols

A reaction between fusel alcohols and Acetyl-CoA and Acyl CoA in *Saccharomyces cervisiae* uses ester synthase enzymes, such as alcohol acetyltransferase (AATase), to produce esters. Fusel alcohols are a mixture of many alcohols that are produced during fermentation. Both the type of fusel alcohol and strain of yeast factor into which esters will be produced during fermentation. Esters are essential to the final flavor determination of the beer and with many factors affecting their production, problems can occur in a hurry. Low ester production could cause a poor flavor profile and cost brewers money and time if the beer is unusable and cannot be sold. This research has been done in an attempt to compare yeast strains with similar types of produced esters, resulting in similar flavor profiles, and then factor in other characteristics such as price. This will allow the determination of which yeast, or combination of yeasts, would be most cost effective, resistant to diseases or temperature changes, and still give the same flavor profile. Several experimental setups were constructed in attempt to efficiently produce and characterize esters. *S. cerevisiae* was incubated with a fusel alcohol in aerobic conditions for 12-24 hours. The alcohols and esters were then extracted with pentane for gas chromatography mass spectroscopy (GC-MS) analysis. Data analysis has shown that we cannot jump into the pathway at this point, but instead must back up and try to influence fusel alcohol production at earlier points in the pathway. Most samples have shown little to no esters that can clearly be identified

from their precursor alcohol. Research is currently being done, and will continue in the future, in order to find the best experimental method for ester production. Once that is accomplished, different strains of yeast will be tested to see what esters are produced by the different strains.

References:

1. Verstrepen, Kevin J., Derdelinckx, Guy, Dufour, Jean-Pierre, Winderickz, Joris, Thevelein, Johan M., Pretorius, Isak S., and Delvaux, Freddy R. "Flavor Active Esters: Adding Fruitiness to Beer." *Journal of Bioscience and Bioengineering* 96, no. 2 (2003): 110–118.
2. Ehrlich, Steven D. "Brewer's Yeast." *University Maryland Medical Center* (2011).
3. Hazelwood, Lucie A., Daran, Jean-Marc, van Maris, Antonius J.A., Pronk, Jack T., and Dickinson, J. Richard. "The Ehrlich Pathway for Fusel Alcohol Production: a Century of Research on Saccharomyces Cerevisiae Metabolism." *Applied and Environmental Microbiology* 74, no. 8 (2008): 2259–2266.

Production of esters by *Saccharomyces cerevisiae* fermentation of different fusel alcohols

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DANIELLE SHERMAN

Content

- Goal
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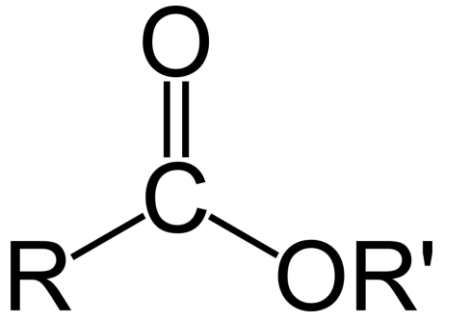
- Experimental Methods
- Results
- Current and Future Work
- Conclusions
- Questions

Introduction to Research

- Goal:
  - Compare yeast strains with similar types of produced esters, resulting in similar flavor profiles.
  - Determine which of these yeast, or combination of yeasts, has the best desirable characteristics overall

Background

- Esters are what give beers their fruity and floral tones[1]
- Ester production is affected by yeast strain, temperature, genetic modifications, and other factors[1]
- Fusel alcohols are a combination of many alcohols, specifically butanol and amyl alcohol isomers[3]



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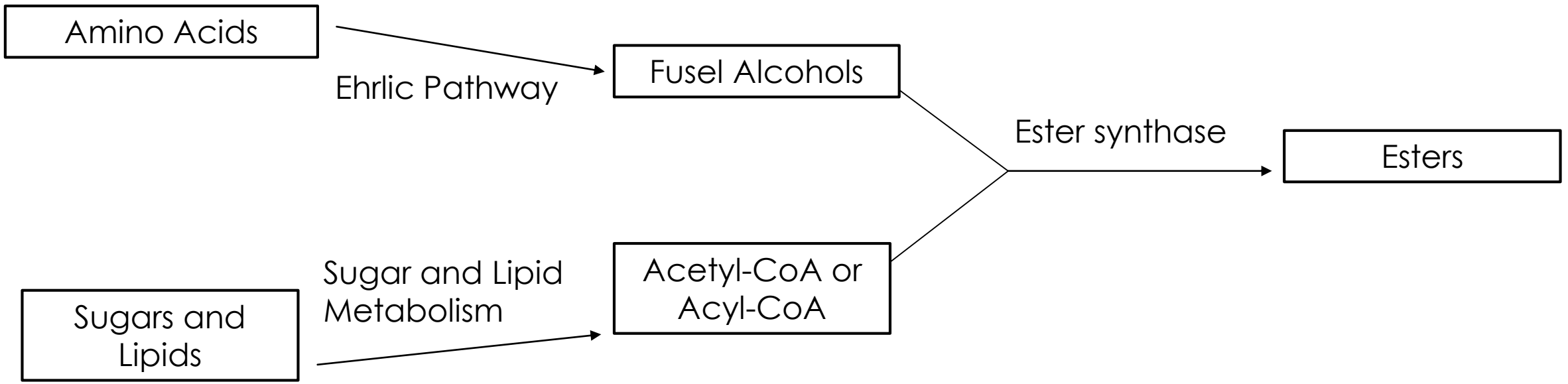
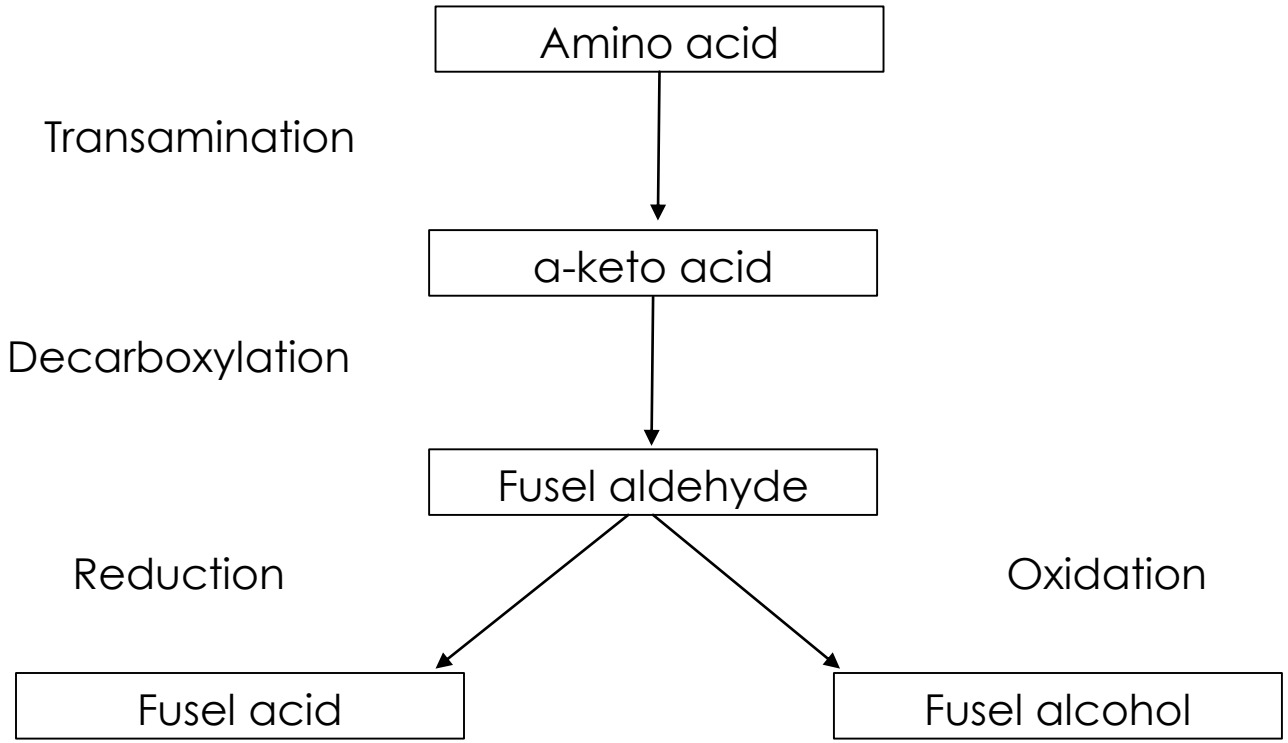

Amino Acids
Ehrlic Pathway
Fusel Alcohols
Ester synthase
Esters
Sugars and Lipids
Sugar and Lipid Metabolism
Acetyl-CoA or Acyl-CoA

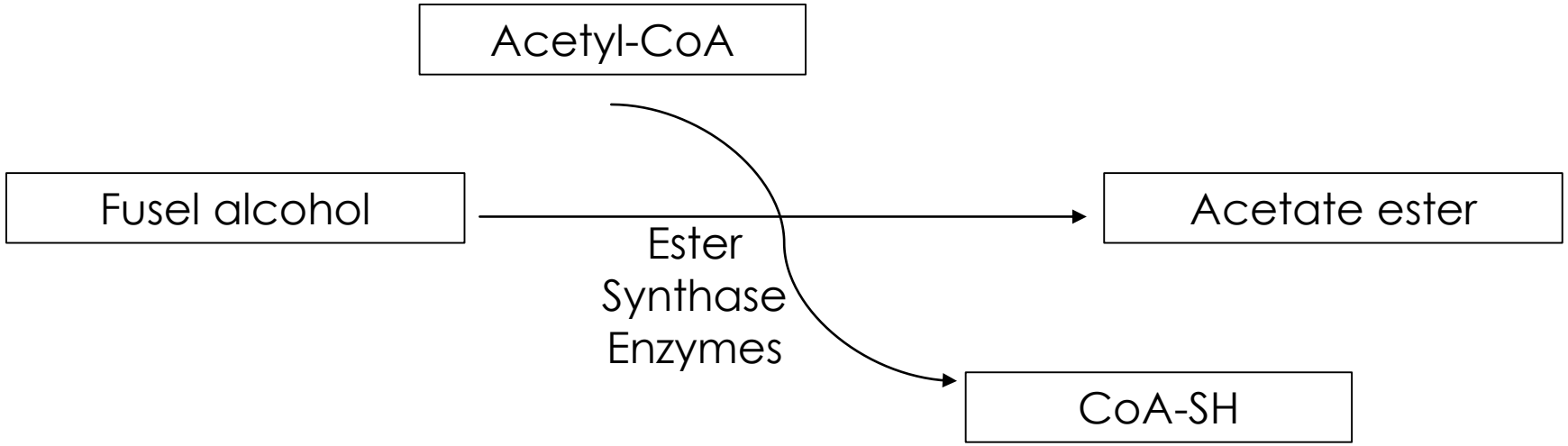
Background

- Amino acid catabolism via the Ehrlic pathway is responsible for the fusel alcohols. [2]



Background

- Fusel alcohols and Acetyl-CoA and Acyl CoA use the ester synthase enzymes such as alcohol acetyltransferase (AATase) to produce esters.[5]



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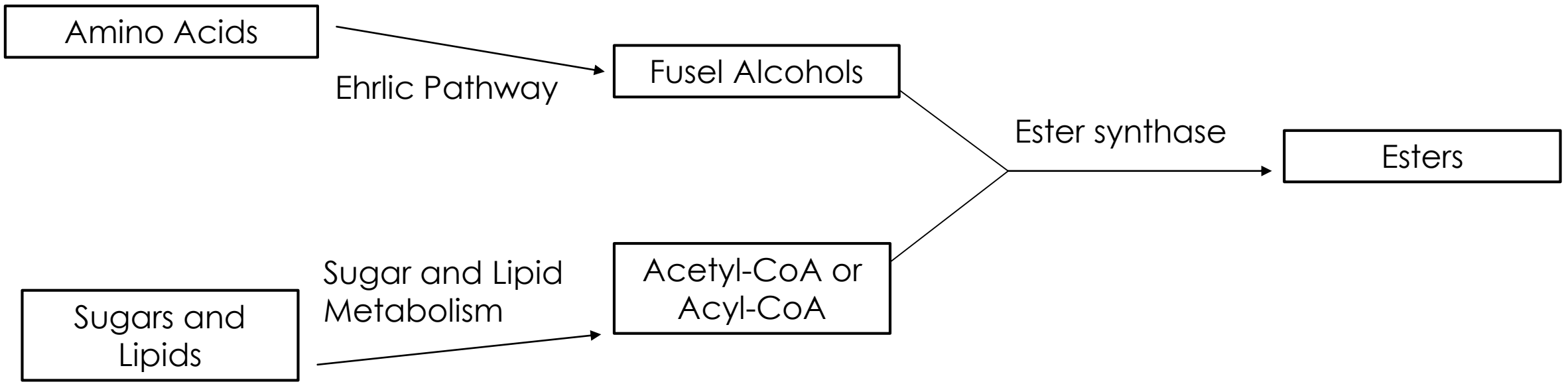
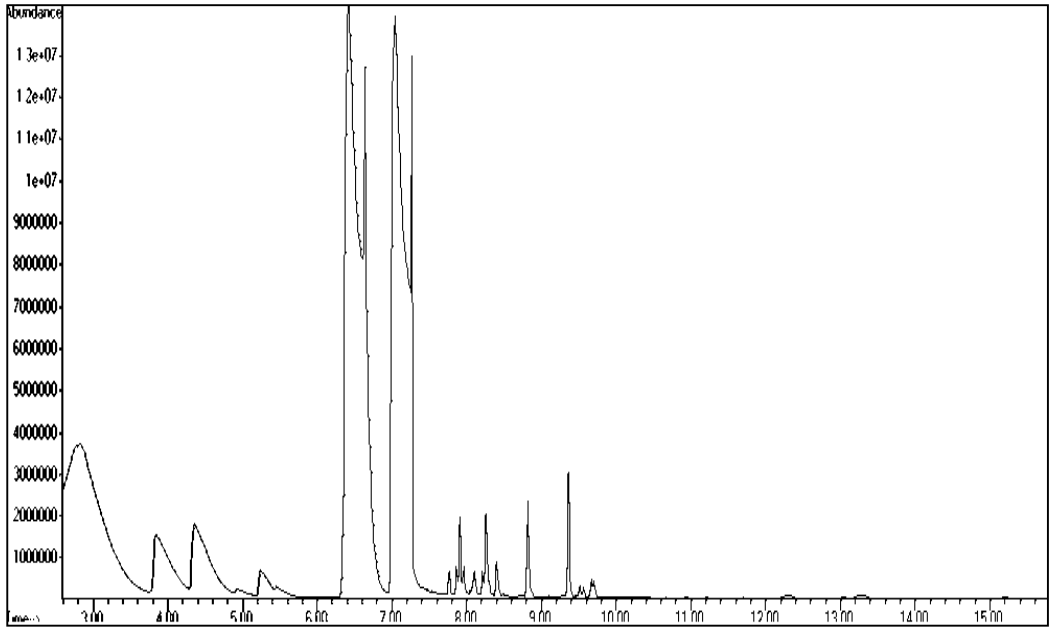

Amino Acids
Ehrlic Pathway
Fusel Alcohols
Ester synthase
Esters
Sugars and Lipids
Sugar and Lipid Metabolism
Acetyl-CoA or Acyl-CoA

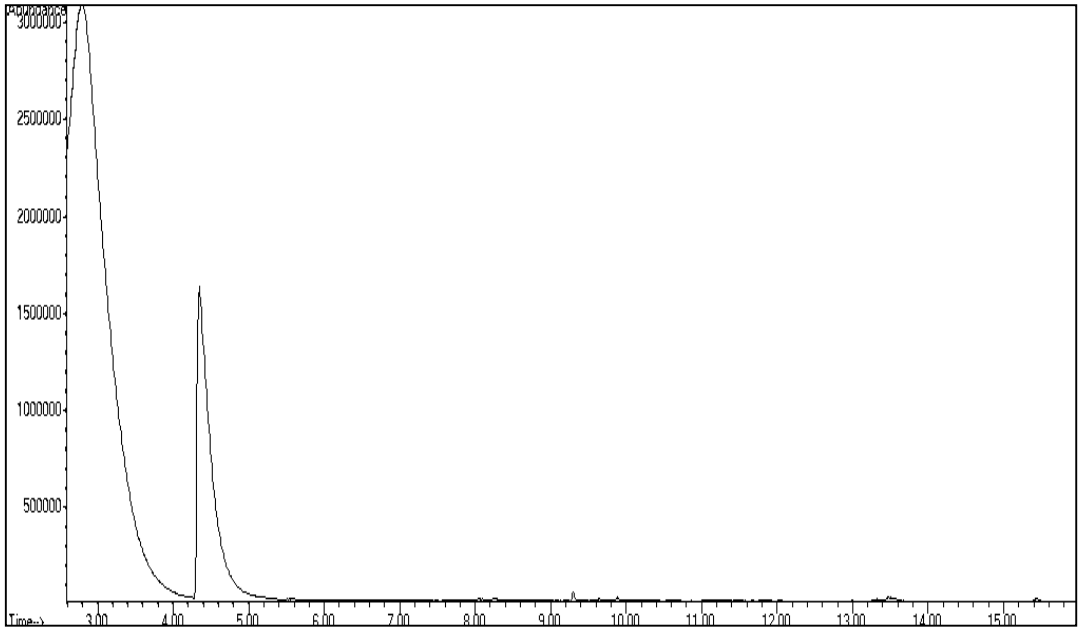
Experimental Procedures

- Yeast was allowed to ferment with a known fusel alcohol for a time ranging from hours to days in a synthetic media
- 250mL flasks were autoclaved and 100mL synthetic media, 1mM acetate, 0.5g yeast, 0.5mM concentration of fusel alcohol, and a pentane overlay were added to the flask.
- Solutions were disturbed with airflow
- The pentane overlay was analyzed using GC-MS

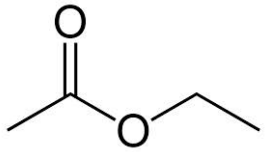
Results

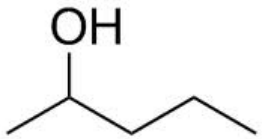


Pentyl ester GC chromatograph



Pentanol GC chromatograph





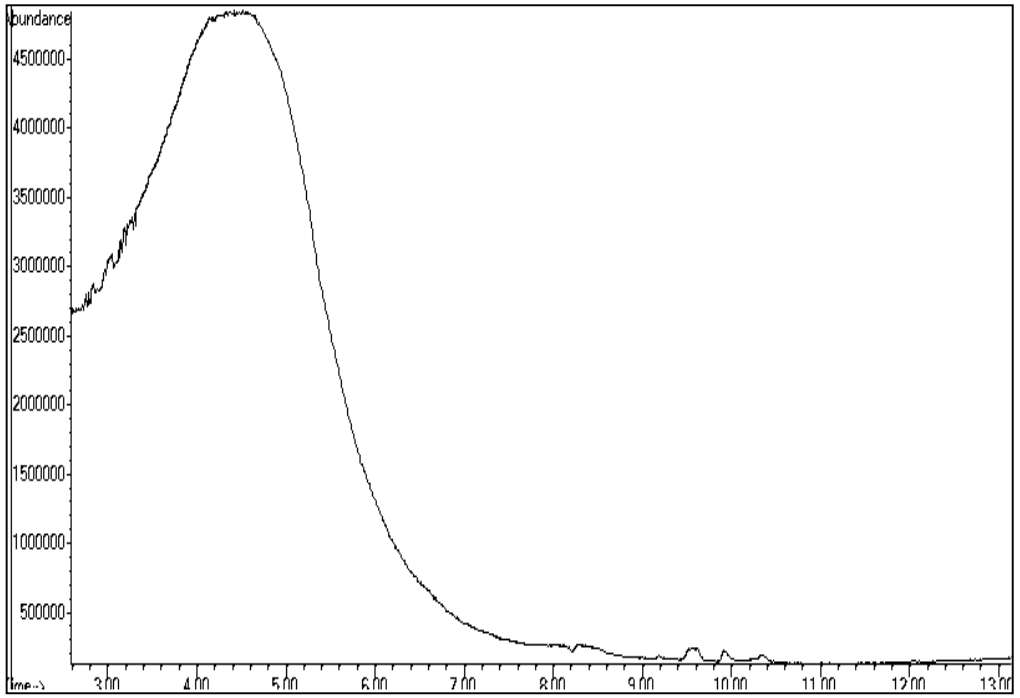
Results

- There are two reasons why are not seeing our desired ester compounds
  - 1. Volatility
  - 2. The yeast is not using the added fusel alcohol

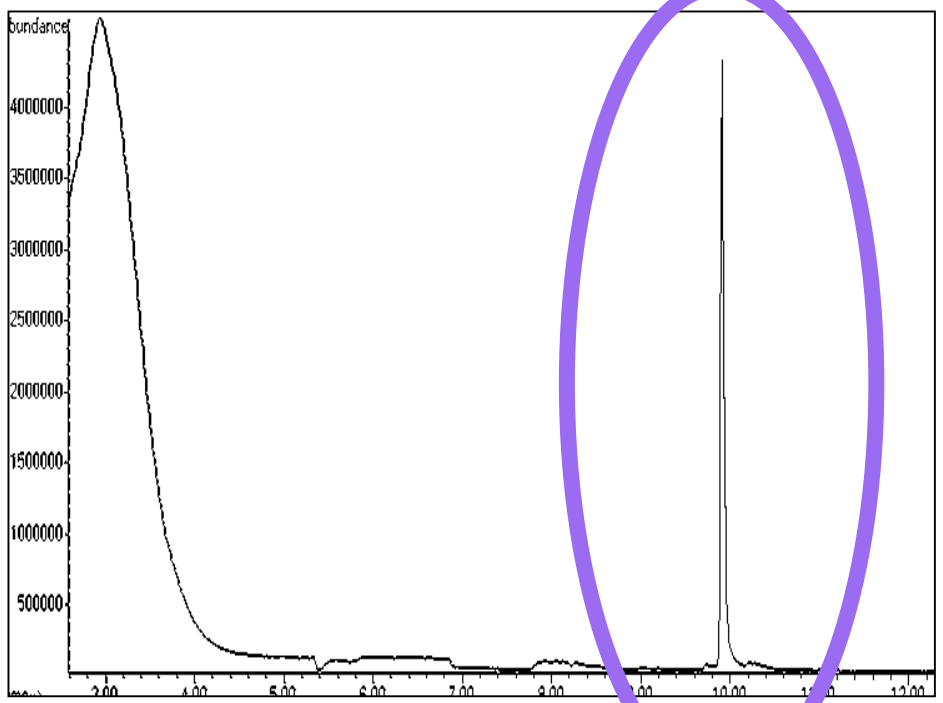
Experimental methods

- 0.100g yeast was fermented with 0.0167g yeast nitrogen base, 0.200g amino acids, 0.200g glucose in 10mL solution
- The samples were bubbled with N_2 gas for 2 minutes
- After, the solutions were left to ferment on shaker table for 3-5 days.

Results

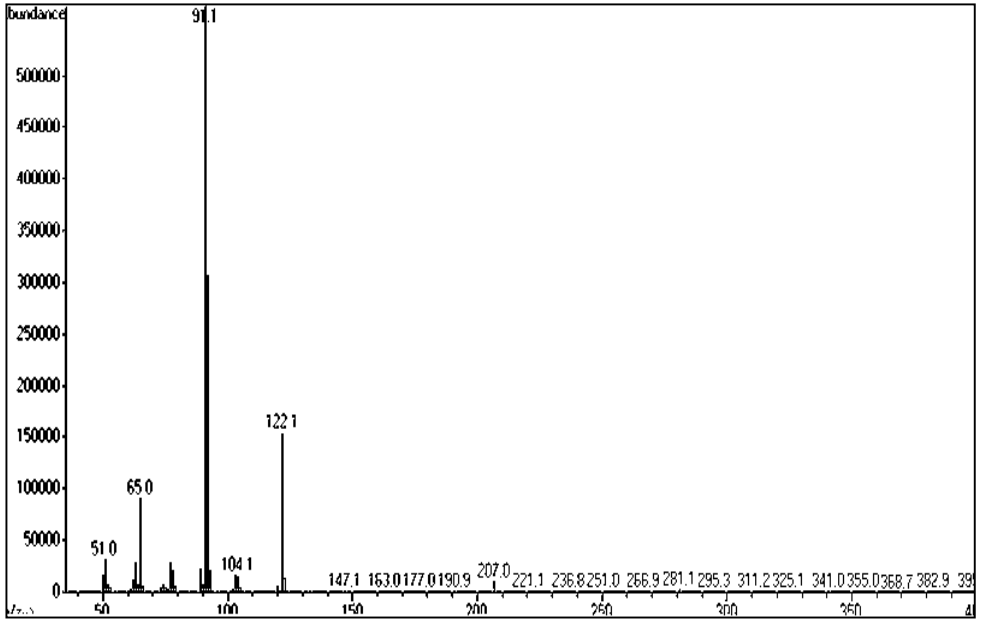


Yeast Nitrogen Base GC spectrum

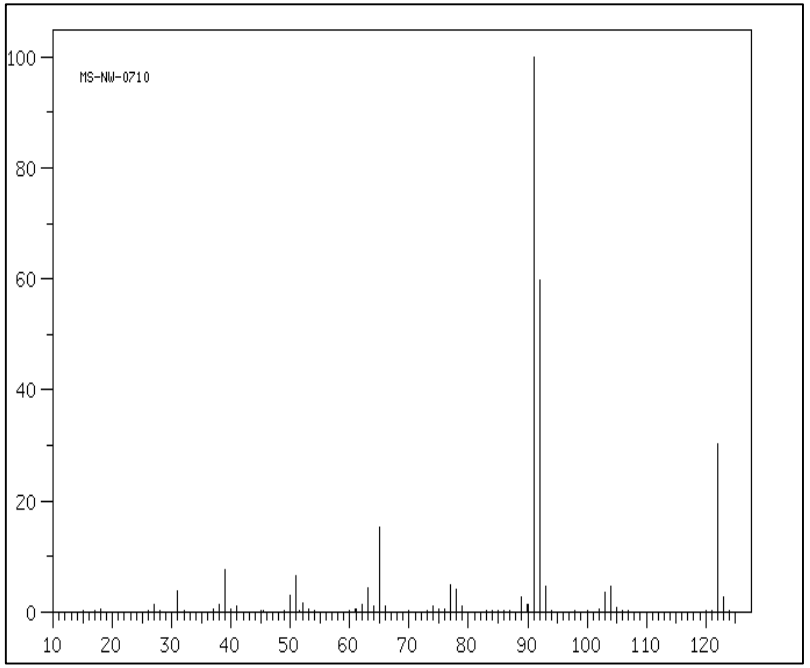


Phenylalanine GC spectrum

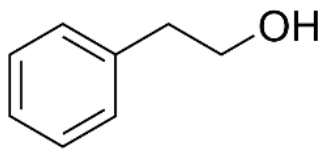
Results



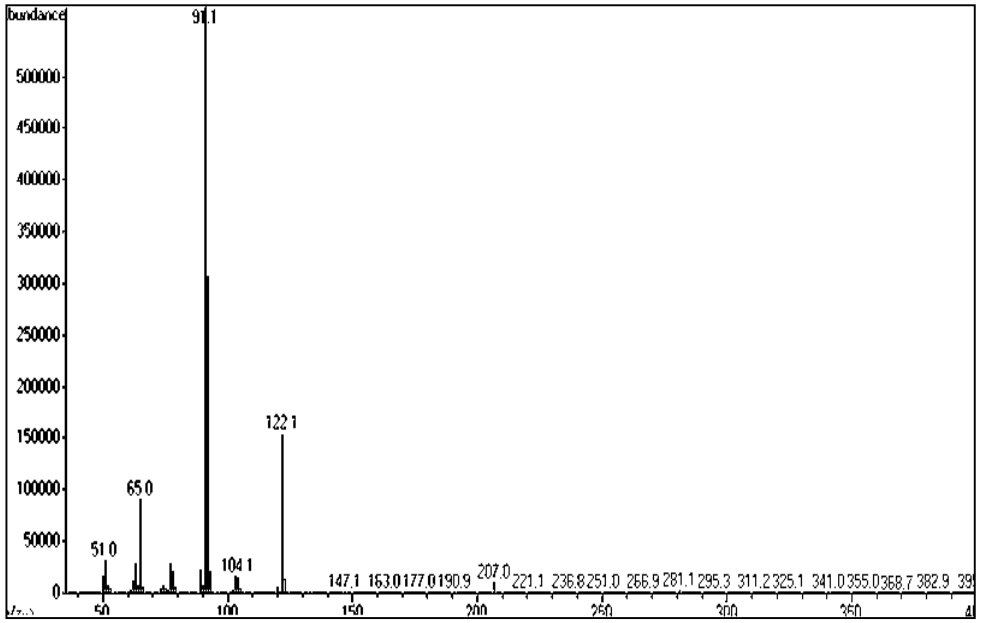
Phenylethyl alcohol MS. Retention time 9.939 min



Literature MS for phenylethyl alcohol



Results



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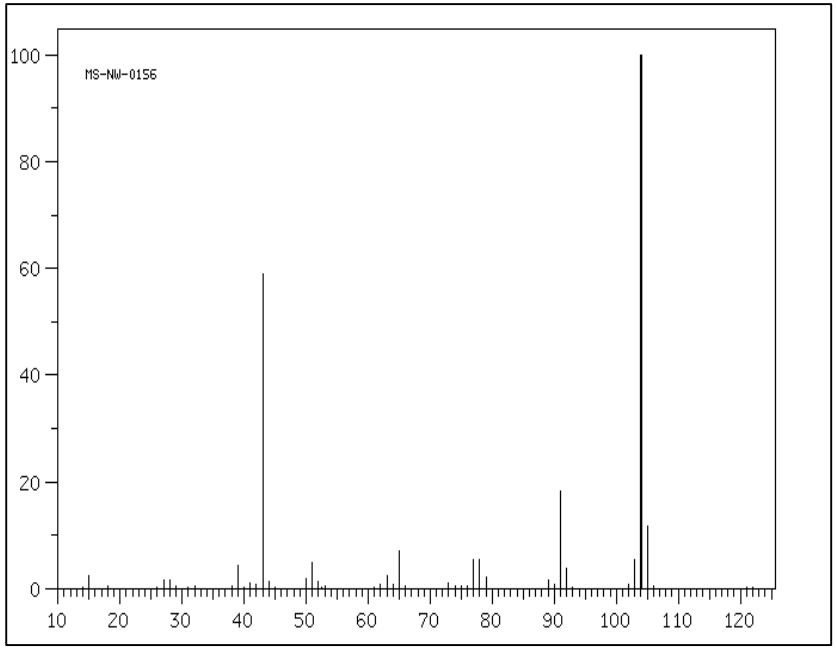

Literature MS phenylethyl acetate

Going Forward

- Fusel alcohol synthesis using a variety of different yeasts and four branched amino acids
- Extract the resulting fusel alcohols with pentane and analyze using GC-MS
- Track the fusel alcohols synthesized by the yeast into their corresponding esters

Conclusion

- The ester synthesis pathway is more complicated than originally thought
- Fusel alcohol production by amino acid degradation has so far proven successful
- There is more work to be done before the final goal can be reached

References

- Verstrepen, Kevin J., Derdelinckx, Guy, Dufour, Jean-Pierre, Winderickz, Joris, Thevelein, Johan M., Pretorius, Isak S., and Delvaux, Freddy R. "Flavor Active Esters: Adding Fruitiness to Beer." *Journal of Bioscience and Bioengineering* 96, no. 2 (2003): 110–118.
- Ehrlich, Steven D. "Brewer's Yeast." *University Maryland Medical Center* (2011).
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- Bekatorou, Argyo, Psarianos, Costas, and Koutinas, Athanasios, A. "Production of Food Grade Yeasts." *Food Technol. Biotechnol.* 44, no. 3 (2006): 407–415.
- Hazelwood, Lucie A., Daran, Jean-Marc, van Maris, Antonius J.A., Pronk, Jack T., and Dickinson, J. Richard. "The Ehrlich Pathway for Fusel Alcohol Production: a Century of Research on Saccharomyces Cerevisiae Metabolism." *Applied and Environmental Microbiology* 74, no. 8 (2008): 2259–2266.

Questions?