Preliminary GC-MS analysis of the effects of yeast and lactic acid bacteria starter cultures on cacao micro-fermentations.

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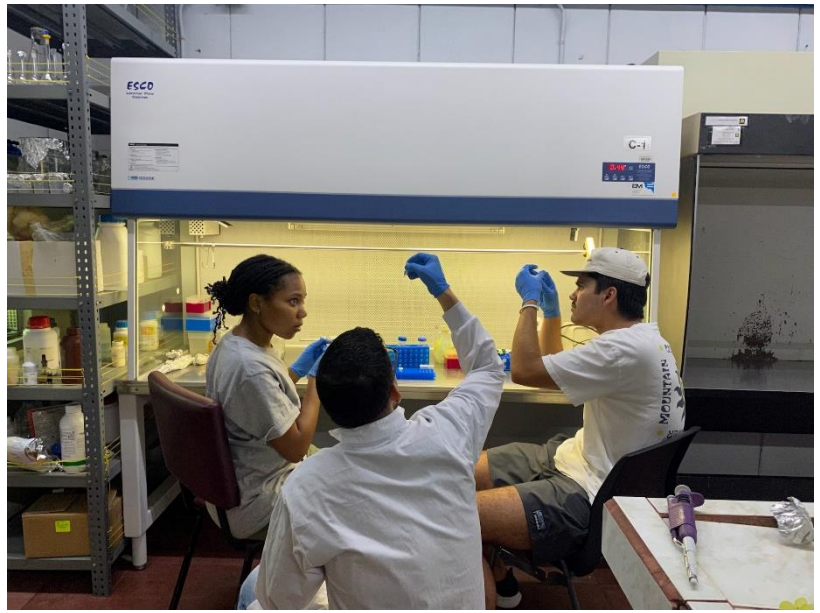
Acknowledgements

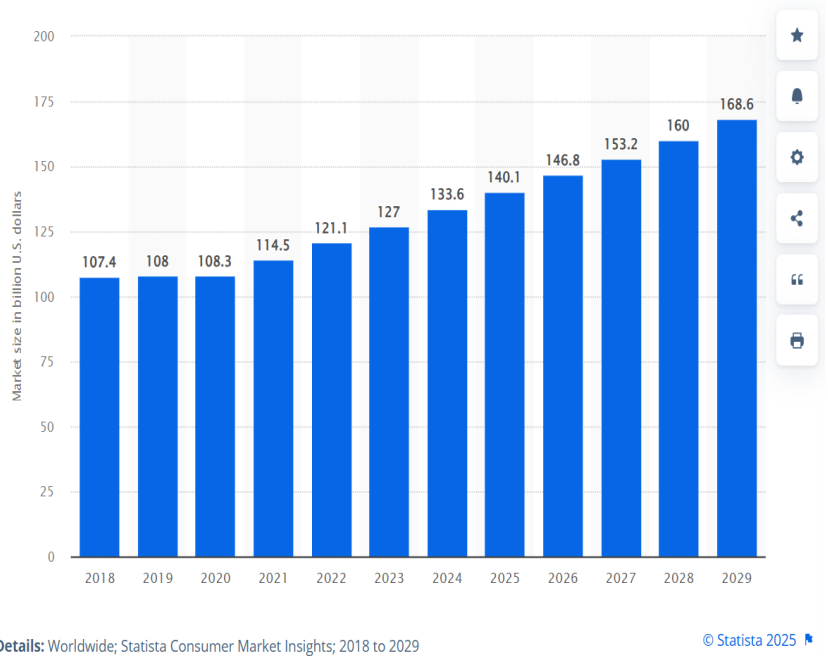
NSF – IRES Award # 2107424 : The Broad Impact of Flavor, A Research Experience on Metabolomics of Microbial Populations during Cacao Fermentation

The IRES a 9-week experience



Context and Problem

- Cacao bean market: $17 billion in 2024
- Chocolate market: $130 billion in 2024
- Premium chocolate market: $37 billion in 2024



- Most cacao is produced by smallholders who live in poverty
- An important portion of the “bulk” chocolate could be premium or ultra-premium, but quality “potential” is lost during fermentation and drying



Harvest

Beans w mucilage

Posthar vest

Export

Ingredients

Process Package

Coverture

Cocoa Butter

Cocoa Powder

Confecti oner

Export

Multiple Farms

Chocolate Manufacturer

Retail

Retail

Retail

Retail

Developing Country

Developed Country

A very complex problem

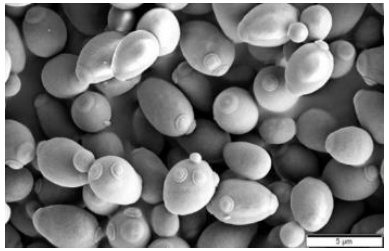
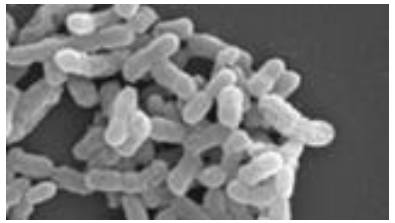






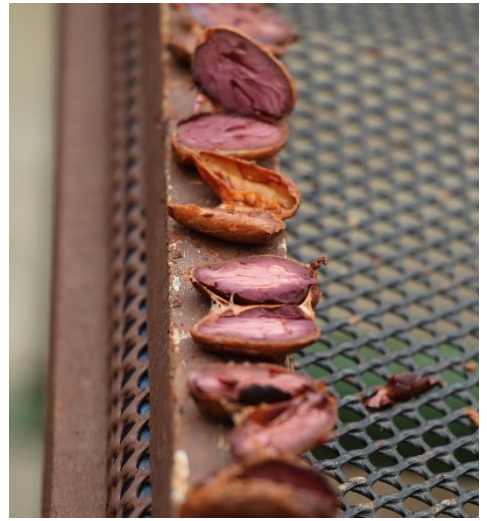

10 major genetic groups


Varying composition
Incl. amount of mucilage and maturity

Varying yeast and bacterial populations

Pathogens

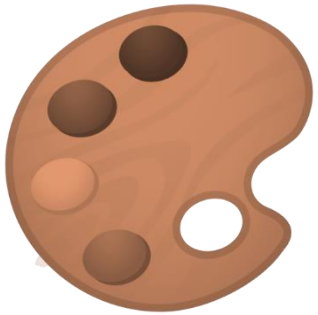



Varying results

Well fermented
Well dried
Fine flavor
From mature fruit

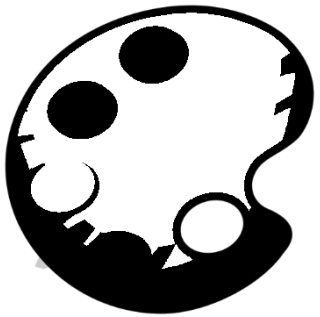
$450

Reasonably fermented
Well dried
Fine flavor
From mostly mature fruit



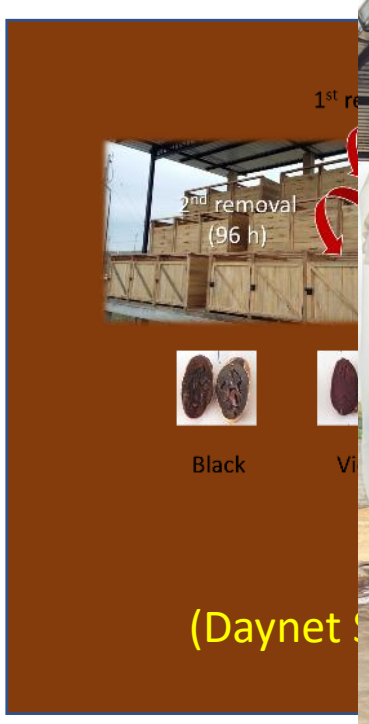
$9

Fermented
Well dried
Any variety
Reasonably mature fruit



$1.32

Cacao Fermentation

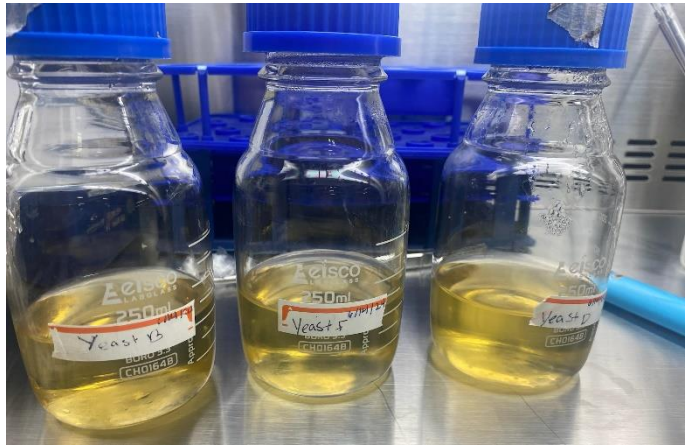


n we design a
rmentor and a
rmentation
otocol that:

Minimizes variability,
Easy to operate,
Affordable, and
Farmers willing to use?

Strategies to reduce variability

- Use starter cultures
  - Select adequate yeasts and bacteria



- Design small-scale fermentors
  - For research
  - For smallholders

Hypothesis and Objective

- **Hypothesis:** Using a yeast starter culture containing endogenous yeast from a local fermentation can be more effective at replicating an aroma profile, reducing defects, and maintaining microbial diversity depending on the time it is added to fermenting cacao beans.
- **Objective:** The objective of this experiment is to elucidate whether the time in which a yeast and a lactic acid bacteria are added to a fermentation (0h vs 12h) will have a significant impact on the resulting aromatic profile *and presence of defects as well as on microbial diversity.*
  - Sample-to-sample variability (replicate)
  - Variability as a function of inoculation time

Experiment Design

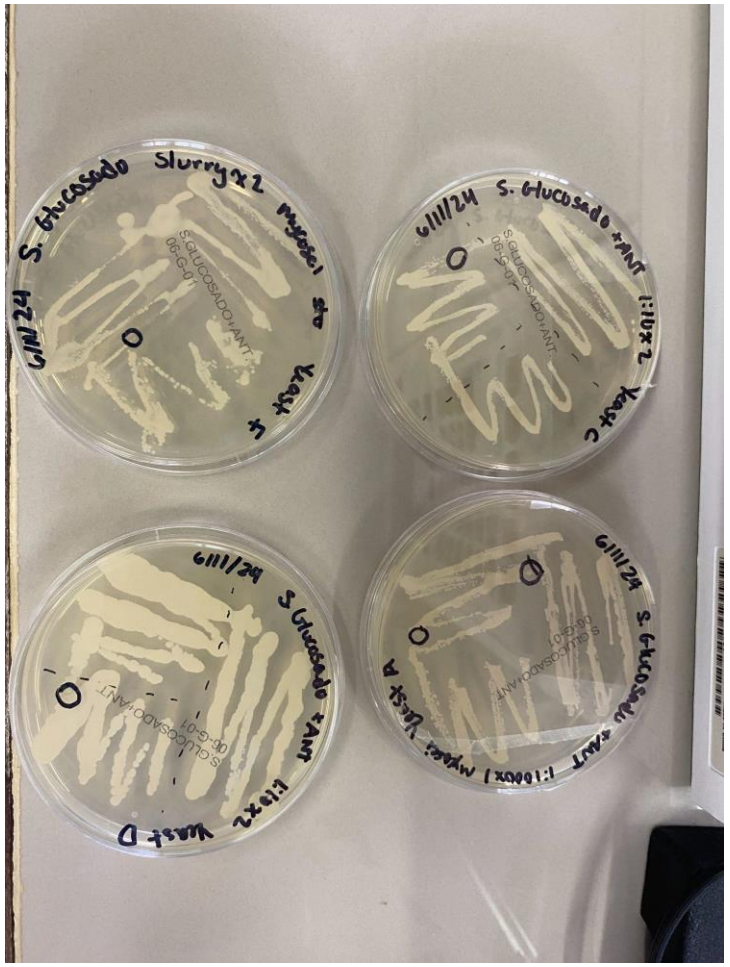
Analyses were done after 24 and 48 h

Starter culture added at 0 h

Starter culture added at 12 h

Yeast Collection

Culturing



Starter culture

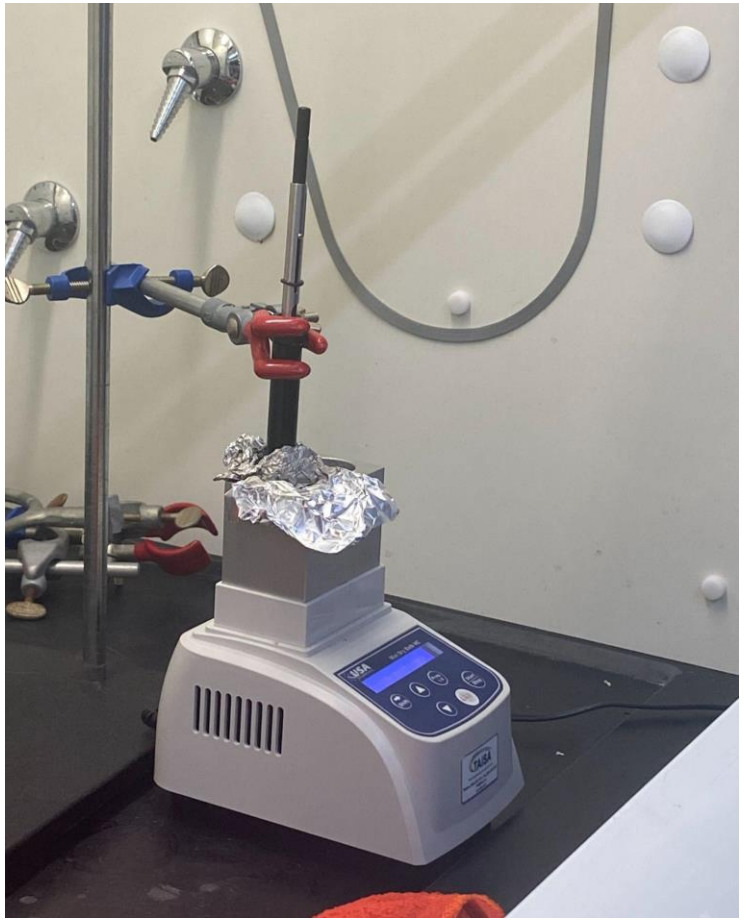
Pod Collection

Sample Prep

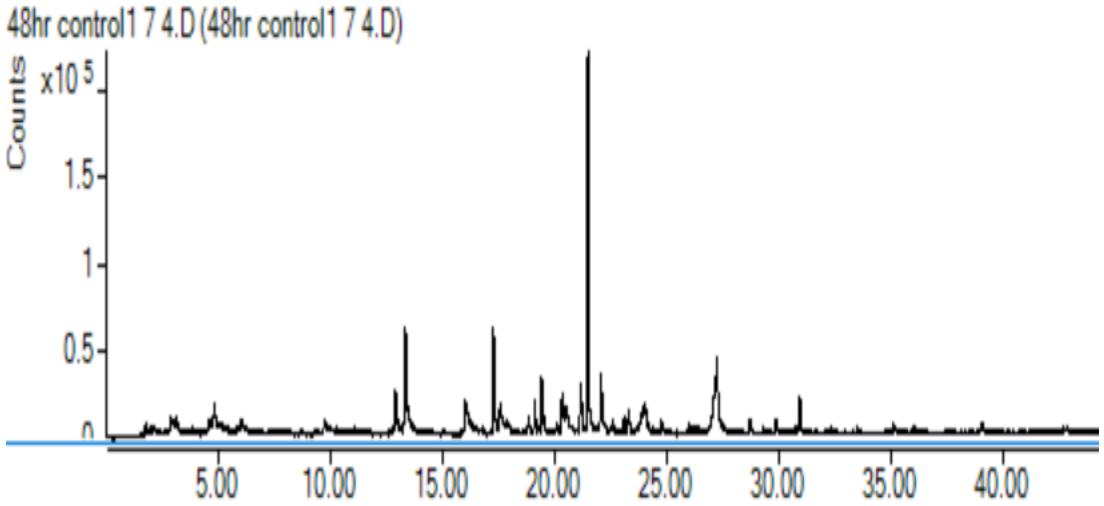
Fermentation

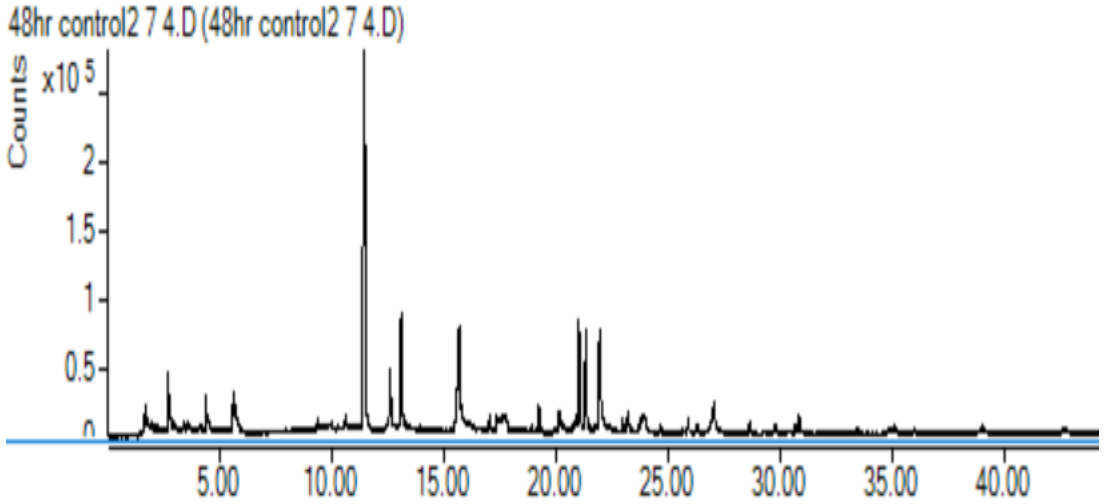
Drying

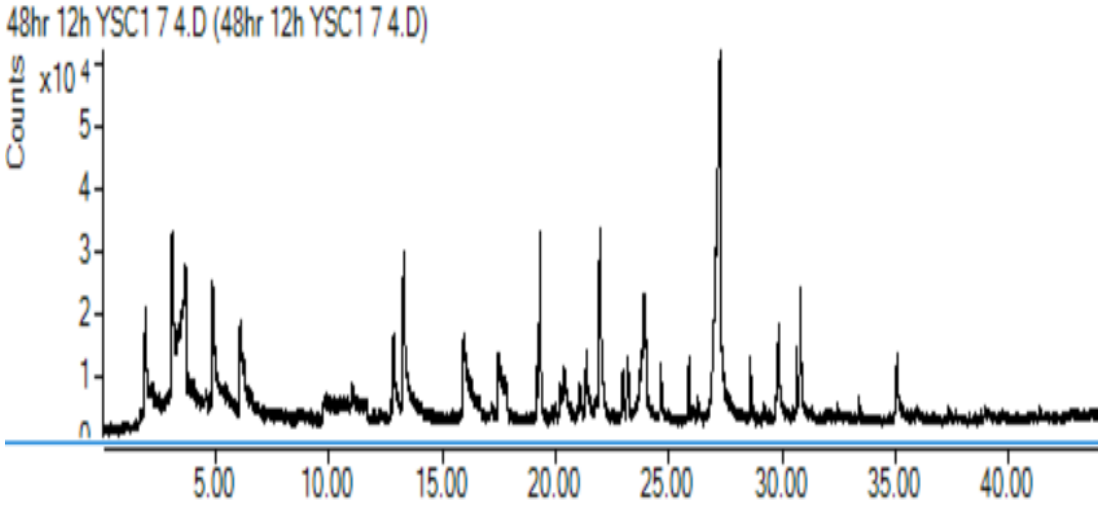
SPME-GC-MS

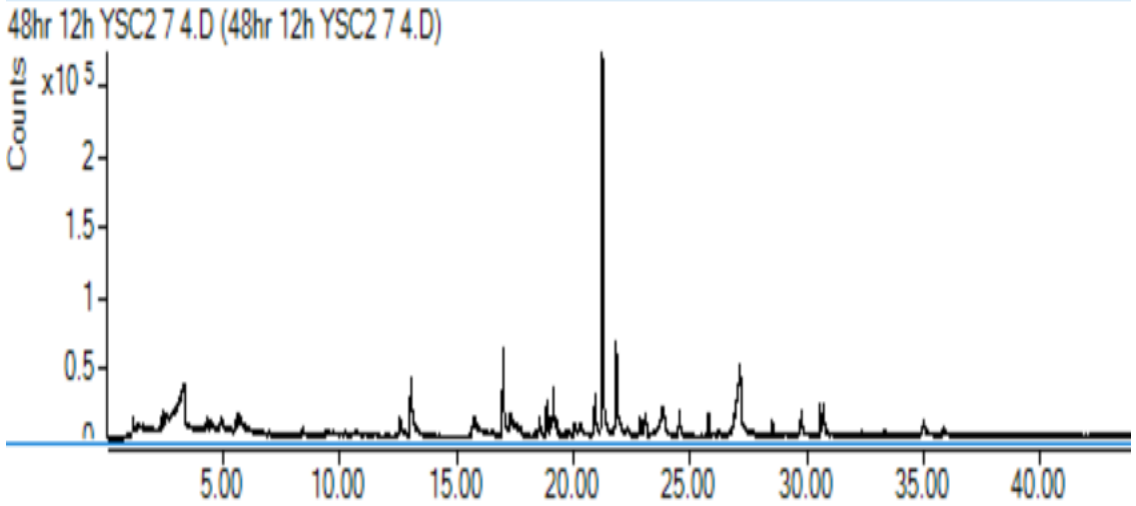


Replicate variability

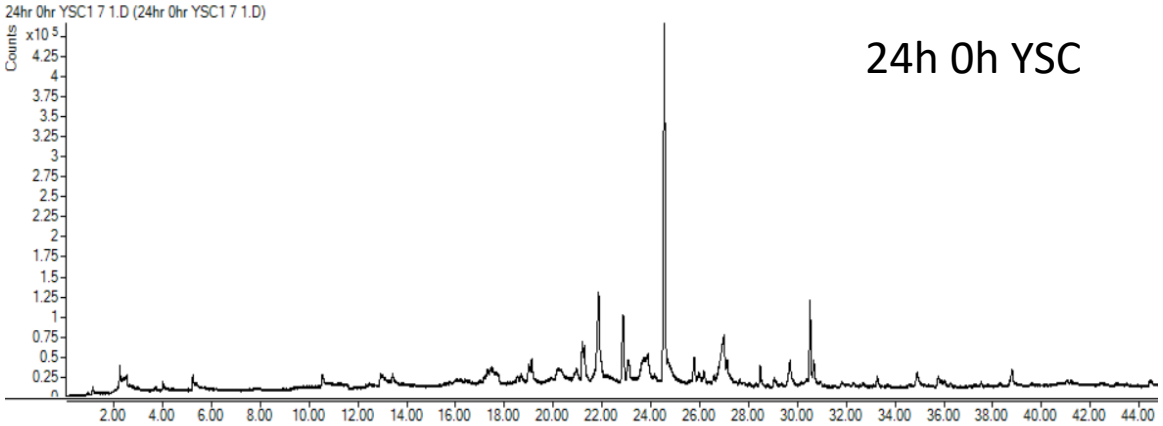




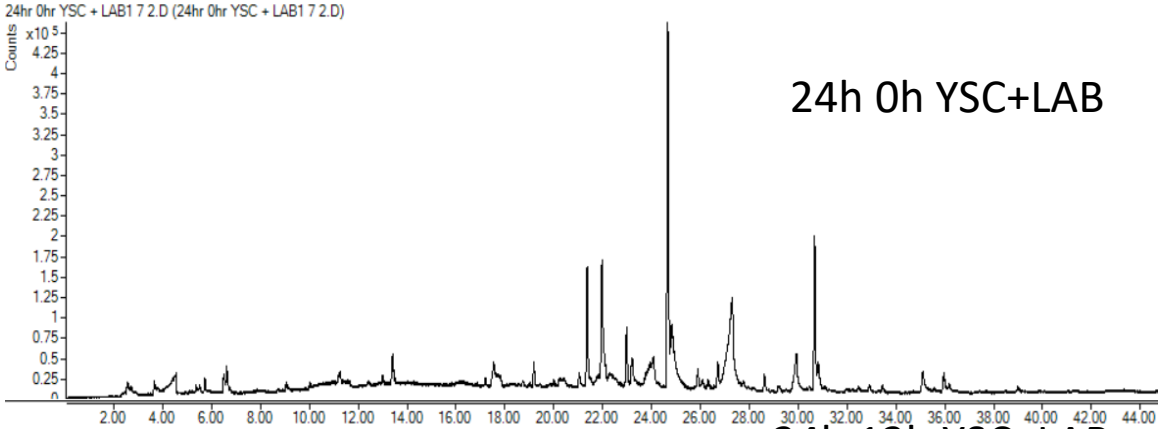




Effect of time of inoculation

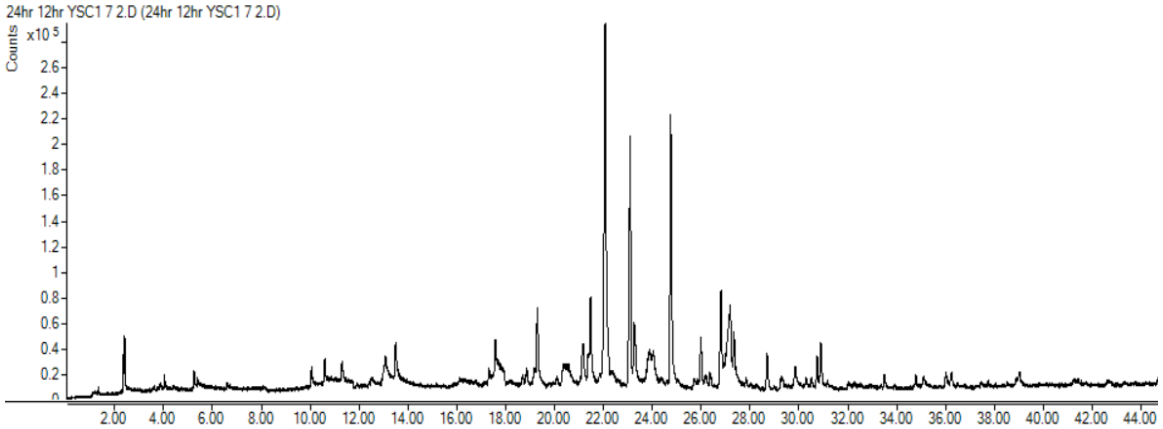


24h 0h YSC

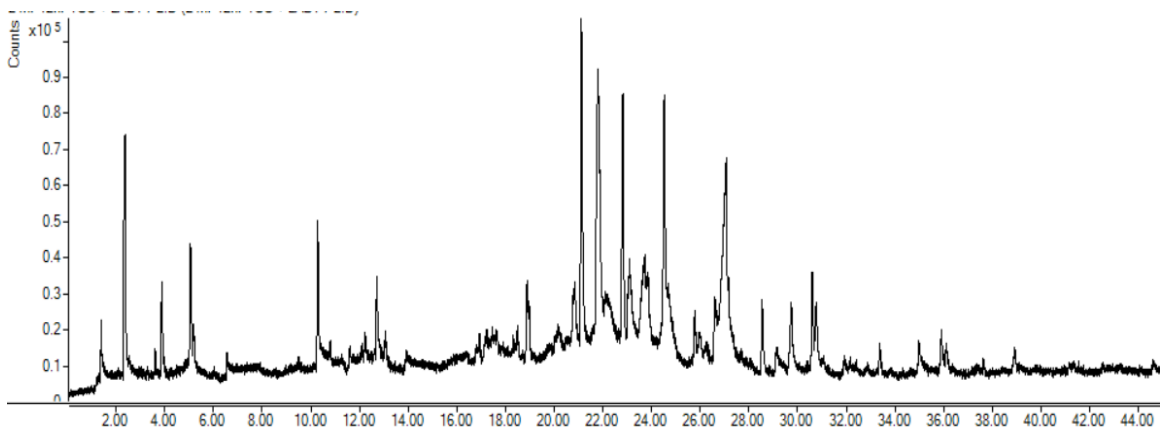


24h 0h YSC+LAB

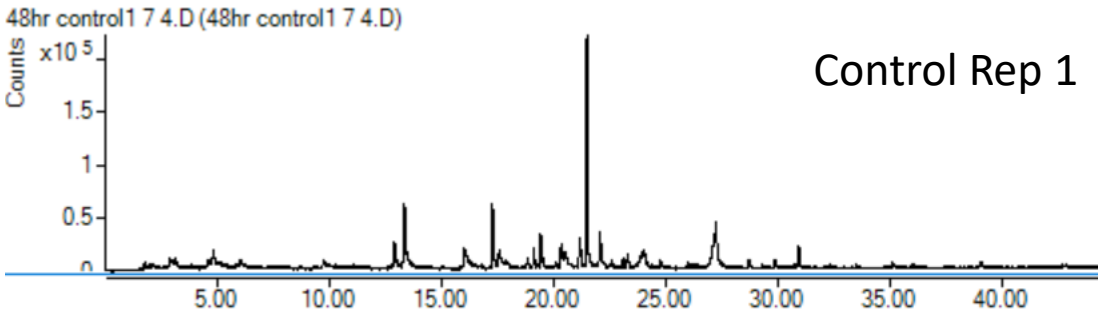
24h 12h YSC



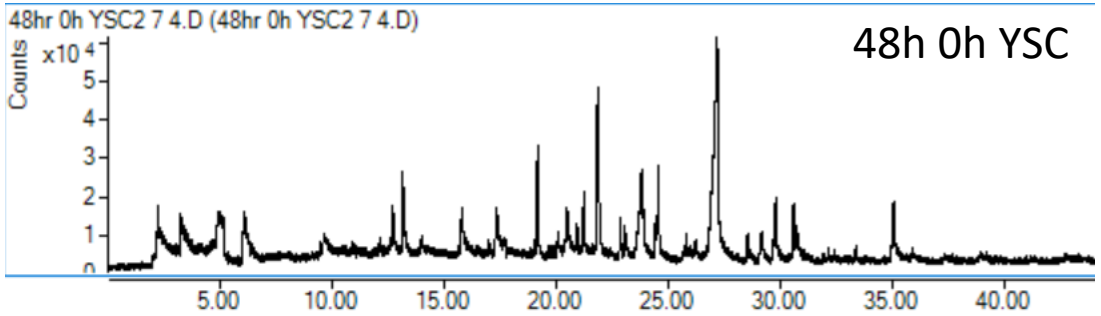
24h 12h YSC+LAB



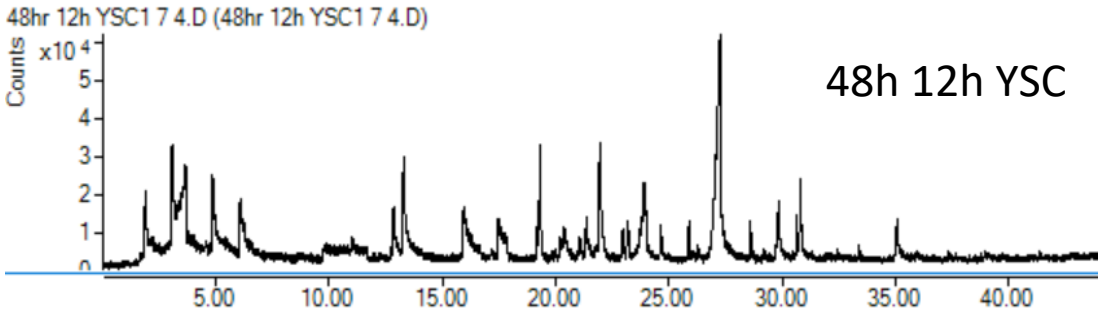
Effect of time of inoculation



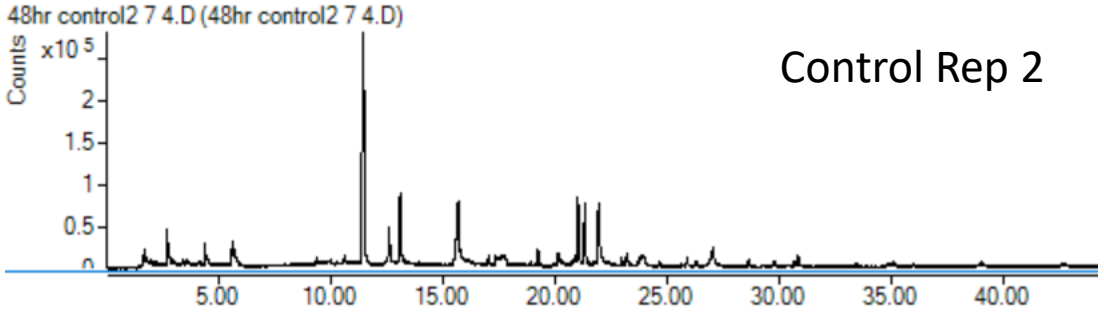
Control Rep 1



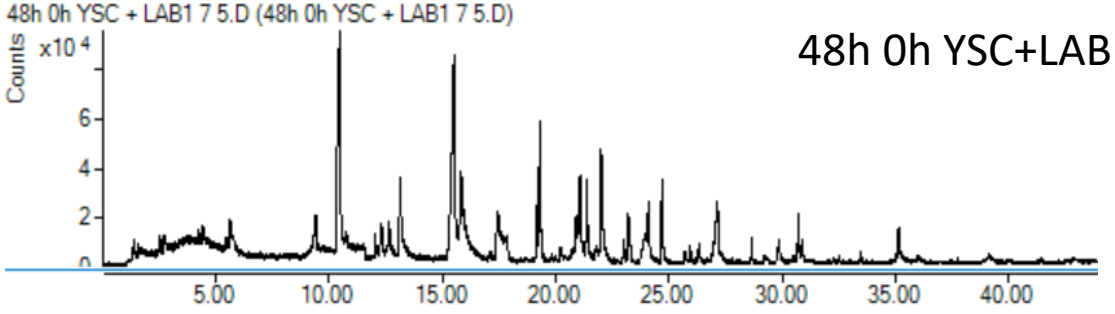
48h 0h YSC



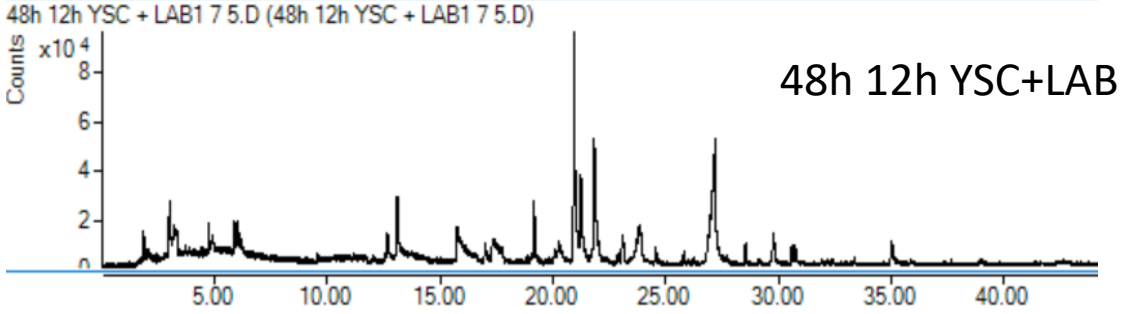
48h 12h YSC



Control Rep 2



48h 0h YSC+LAB

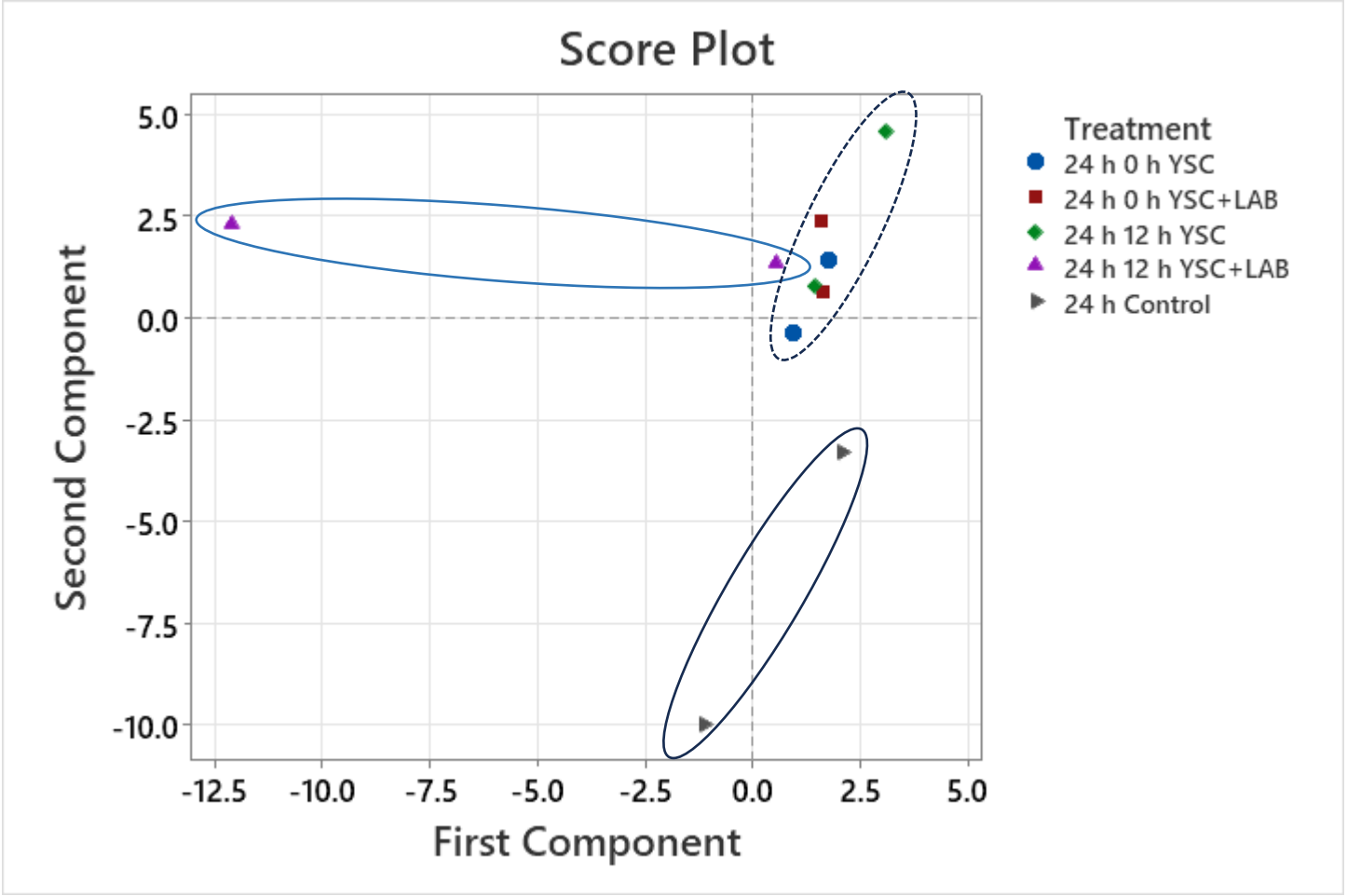


48h 12h YSC+LAB

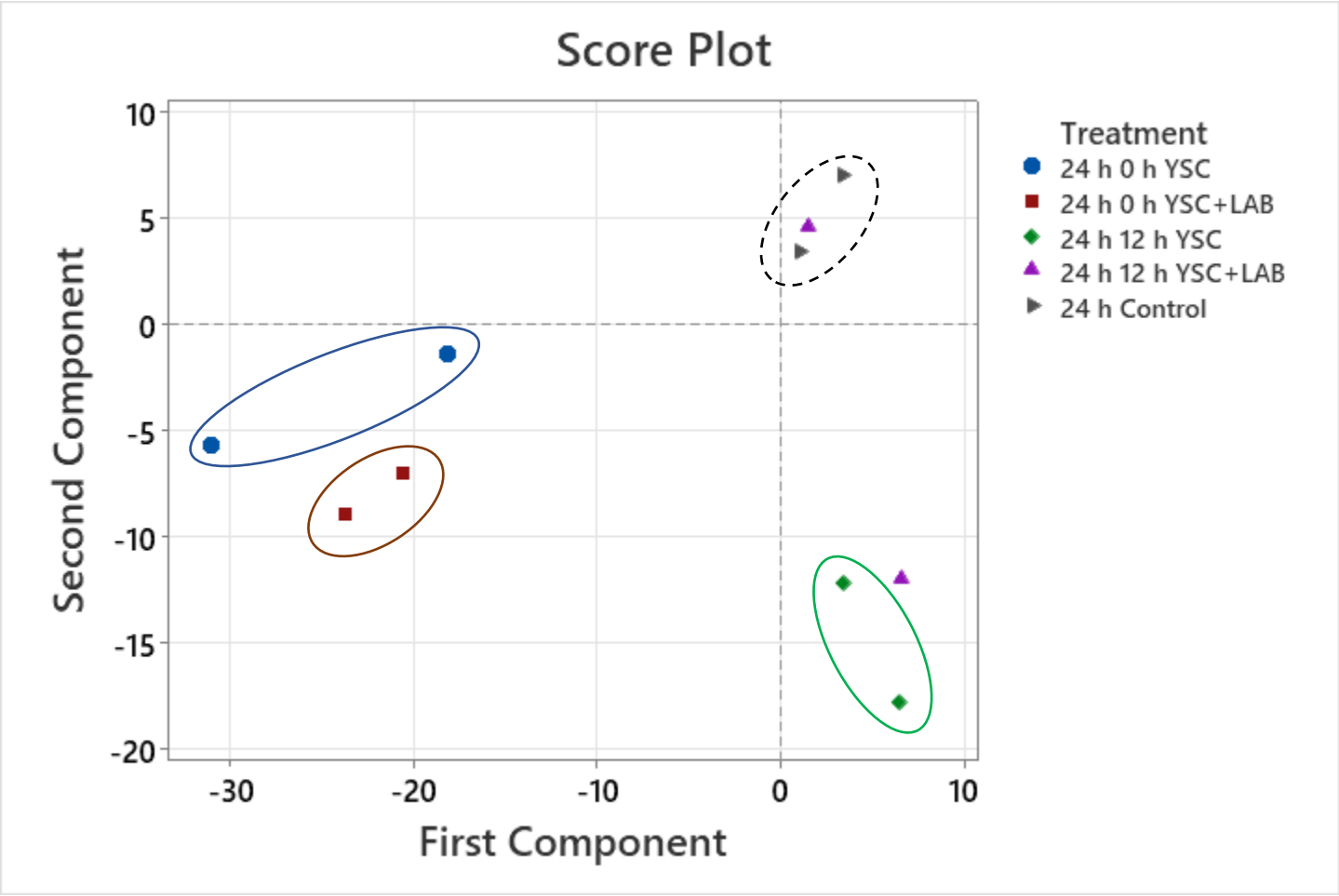
Data Identification & Alignment

Componer	Compound Name	24 h Cont	24 h Cont	24 h 0 h Y	24 h 0 h Y	24 h 0 h Y	24 h 0 h Y	24 h 12 h	24 h 12 h	24 h 12 h	24 h 12 h
2.392531	3-Methoxybenzylamine, N-heptyl-N-octyl-	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.453	2,4(1H,3H)-Pyrimidinedione, 6-iodo-5-methyl- or 3-Buten-2-ol, 2-meth	6.719	6.289	0.000	0.000	0.700	0.000	0.000	0.000	0.000	0.000
3.587972	1-Methyl-1-ethoxycyclobutane	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.174	0.000
4.033899	n-Propyl acrylate	0.000	0.000	0.000	0.000	0.000	0.000	1.150	0.000	0.000	0.000
4.295128	1-Hepten-3-one	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.271
4.490922	2-Propenoic acid, oxiranylmethyl ester	0.000	0.000	0.000	0.085	0.000	0.000	0.000	2.994	0.000	0.000
4.609725	Carbonyl sulfide	0.000	0.000	23.011	24.433	17.608	21.396	0.000	0.000	0.000	0.000
4.838815	1-Methyl-1-ethoxycyclobutane	0.527	0.145	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5.036941	1,5-Hexadiene, 3,3,4,4-tetrafluoro-	0.000	0.000	0.000	0.649	0.000	7.314	0.000	0.000	0.000	0.000
5.129791	2-Propenoic acid, 2-hydroxyethyl ester or 2-Propenoic acid, pentyl este	3.529	3.316	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5.234703	1,2-Oxaborolane, 2-ethyl-4,5-dimethyl-	0.000	0.000	0.000	0.300	0.000	0.000	0.000	0.000	0.000	0.000
5.693821	Oxirane, 2-(1,1-dimethylethyl)-3-methyl-	3.773	5.457	3.797	0.000	0.000	0.000	0.976	5.268	10.171	0.000
5.830416	Methane, isocyanato-	0.000	2.464	0.000	0.000	0.000	0.000	0.000	1.615	0.000	0.000
6.115292	1,2-Oxaborolane, 2-ethyl-4,5-dimethyl-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.535
6.184172	1-Hepten-3-one	0.000	0.000	0.000	0.000	0.000	1.434	0.000	0.000	0.000	0.000
6.336043	3,3-Dimethyl-1,2-epoxybutane	0.000	0.000	0.000	0.000	0.000	3.471	0.000	0.000	0.000	0.000
6.583149	1,3-Benzenediol, 5-iodo-	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000
6.62025	Formic acid, 2-methylbutyl ester	0.000	0.000	0.000	0.000	0.290	0.000	0.000	0.000	0.000	0.000
7.082536	1,5-Hexadiene, 3,3,4,4-tetrafluoro-	15.475	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2.420	8.667
7.492579	2-Propenoic acid, anhydride	0.000	0.000	0.395	0.000	0.000	0.000	0.000	0.000	0.000	0.000
8.169961	1,5-Hexadiene, 3,3,4,4-tetrafluoro-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	4.053	0.000	0.000
9.23754	Carbonocyanidic acid, ethyl ester	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9.505067	Pyridine, 3,5-dichloro-2,4,6-trifluoro-	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.063	0.000	0.000
10.34503	Methanesulfonyl chloride	0.000	0.000	0.000	0.000	0.000	1.428	4.131	0.000	0.000	0.000
10.49563	3-Furaldehyde	0.000	0.000	2.480	0.000	0.000	0.000	0.000	0.000	0.000	0.000
10.59932	[3,3-Bipyrazole]-4,4-dicarboxylic acid, dimethyl ester	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.000	0.000	0.000
11.20269	5-Fluoro-3-trifluoromethylbenzoic acid, ethyl ester	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.533	0.000
11.60175	1H-Imidazole, 2,4-dimethyl-	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000
12.35883	1-Butanol, 3-methyl-, acetate	0.000	0.102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Correlation



Covariance


Score Plot
Second Component
First Component
10
5
0
-5
-10
-15
-20
-30
-20
-10
0
10
Treatment
24 h 0 h YSC
24 h 0 h YSC+LAB
24 h 12 h YSC
24 h 12 h YSC+LAB
24 h Control

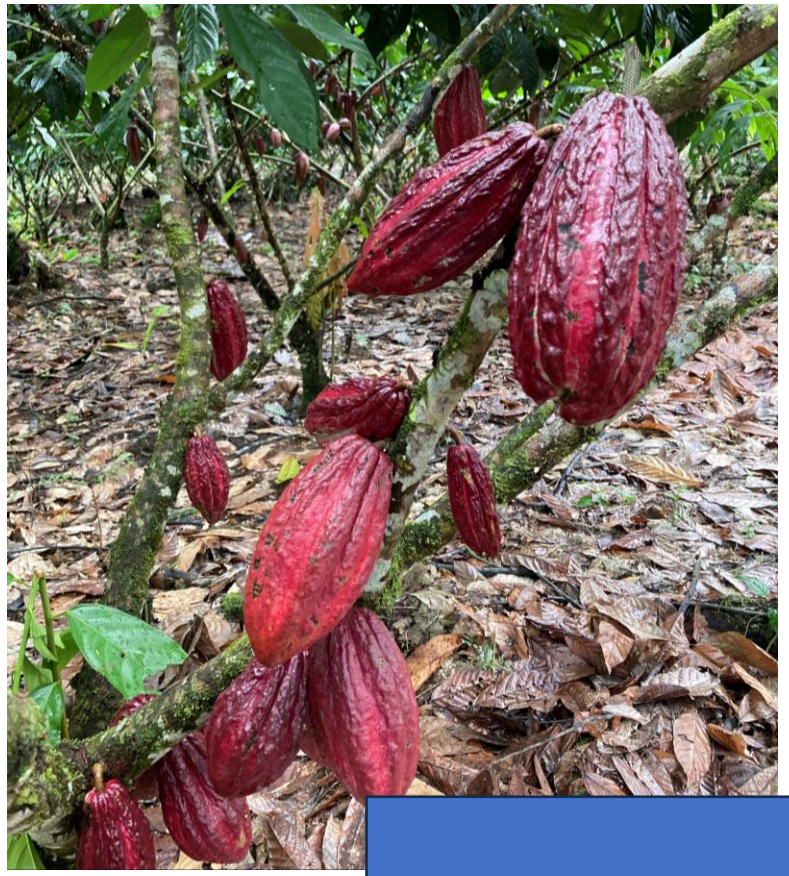
Preliminary Conclusions

- Different inoculums affect the production of volatile composition relative to the control and sampling time.
- Duplicates have important differences in composition
  - Non-uniform inoculation
  - Non-uniform cacao sample distribution – maturity?
- Time of addition has an effect on volatile composition more pronounced when analyzing at 24 h than at 48 h
  - In early inoculation yeast induced more volatiles that yeast + LAB
  - In late inoculation yeast + LAB induced more volatiles than yeast alone

Future work

- Refine compound identification
- Replicate experiment using additional replicates
- Replicate using small (not micro) batches and complete fermentation
- Produce cocoa liquors and assess organoleptic quality

If life gives you



Make

THANK YOU!