Optimizing Cacao Fermentation: The Impact of Yeast Starter Cultures on Microbial Diversity and Flavor Development

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INTRODUCTION

Cacao fermentation is a crucial step in chocolate production, influencing the final product's flavor, aroma, and overall quality. Traditional fermentation relies on natural microbial communities, resulting in high variability and inconsistent flavor profiles, particularly for small-scale farmers. This study explores the use of starter cultures to optimize and control fermentation, with the goal of improving quality and efficiency for small-scale cocoa producers.

Objectives:

- Investigate the impact of yeast starter cultures (YSC) on microbial diversity, particularly lactic acid bacteria (LAB) and acetic acid bacteria (AAB).
- Assess how controlled microbial activity influences flavor and aroma profiles during cocoa fermentation.
- Use molecular and chemical analyses (PCR, DNA sequencing, SPME-GC-MS) to correlate microbial shifts with volatile compound production.
- Provide insights to help small-scale farmers implement more standardized and effective fermentation techniques.

METHODS

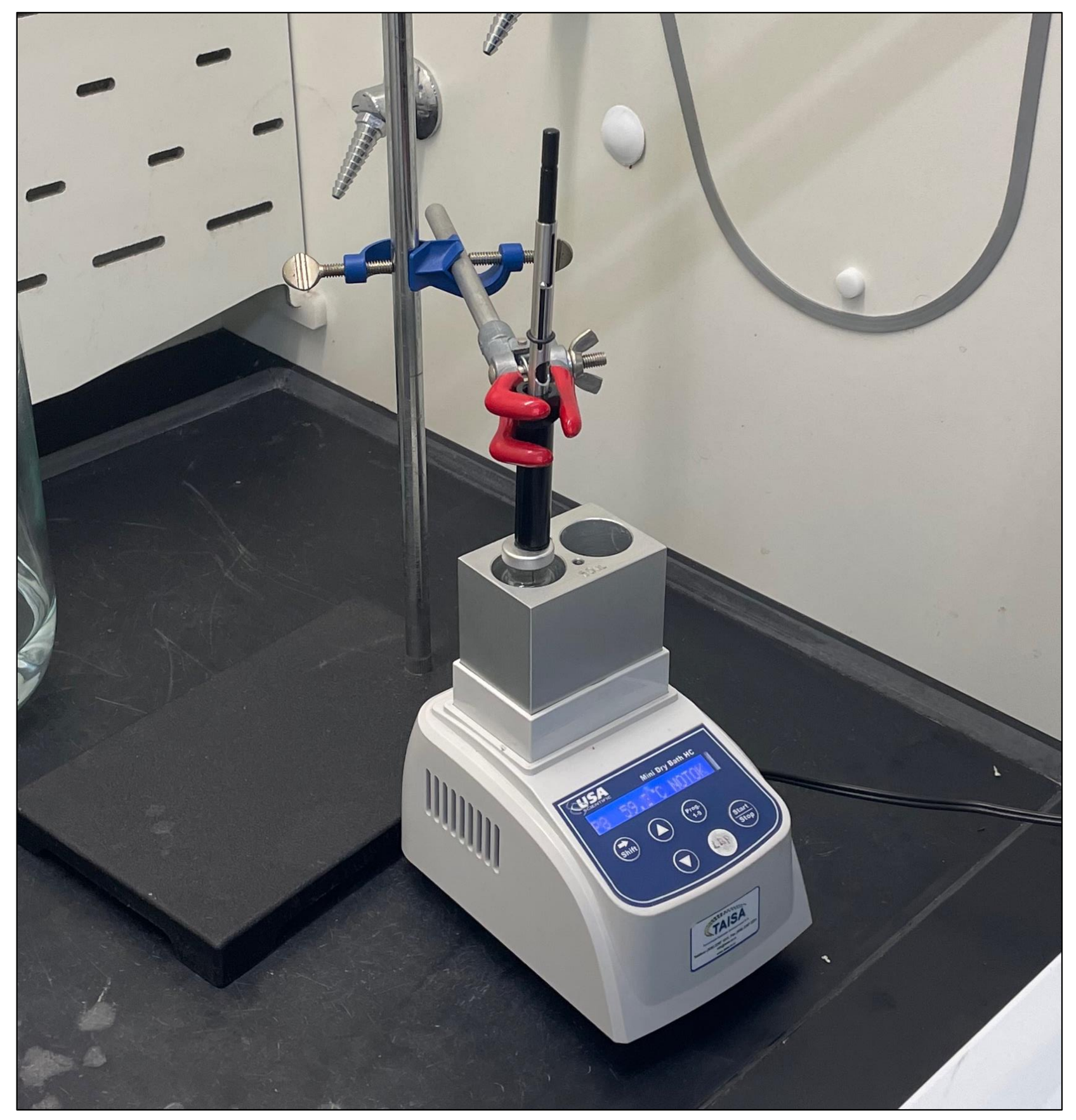
Small-Scale Fermentation and Drying

- 21 jars (1 kg) of cacao beans fermented with/without YSC, LAB, AAB
- 6-day fermentation; samples collected at 24 & 48 hrs
- Beans dried for 11 days with regular rotation

METHODS

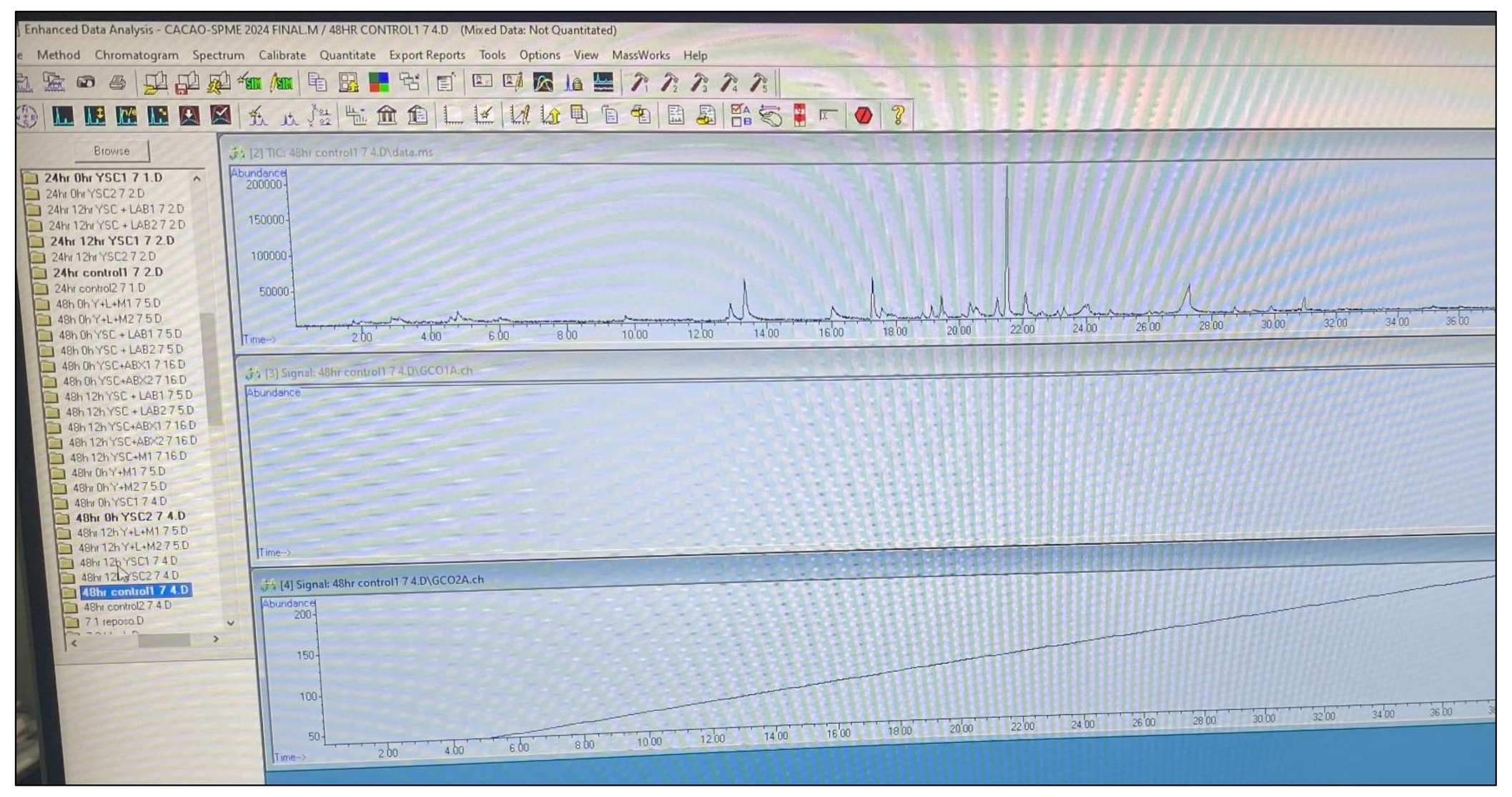
GC-MS (Volatile Analysis)

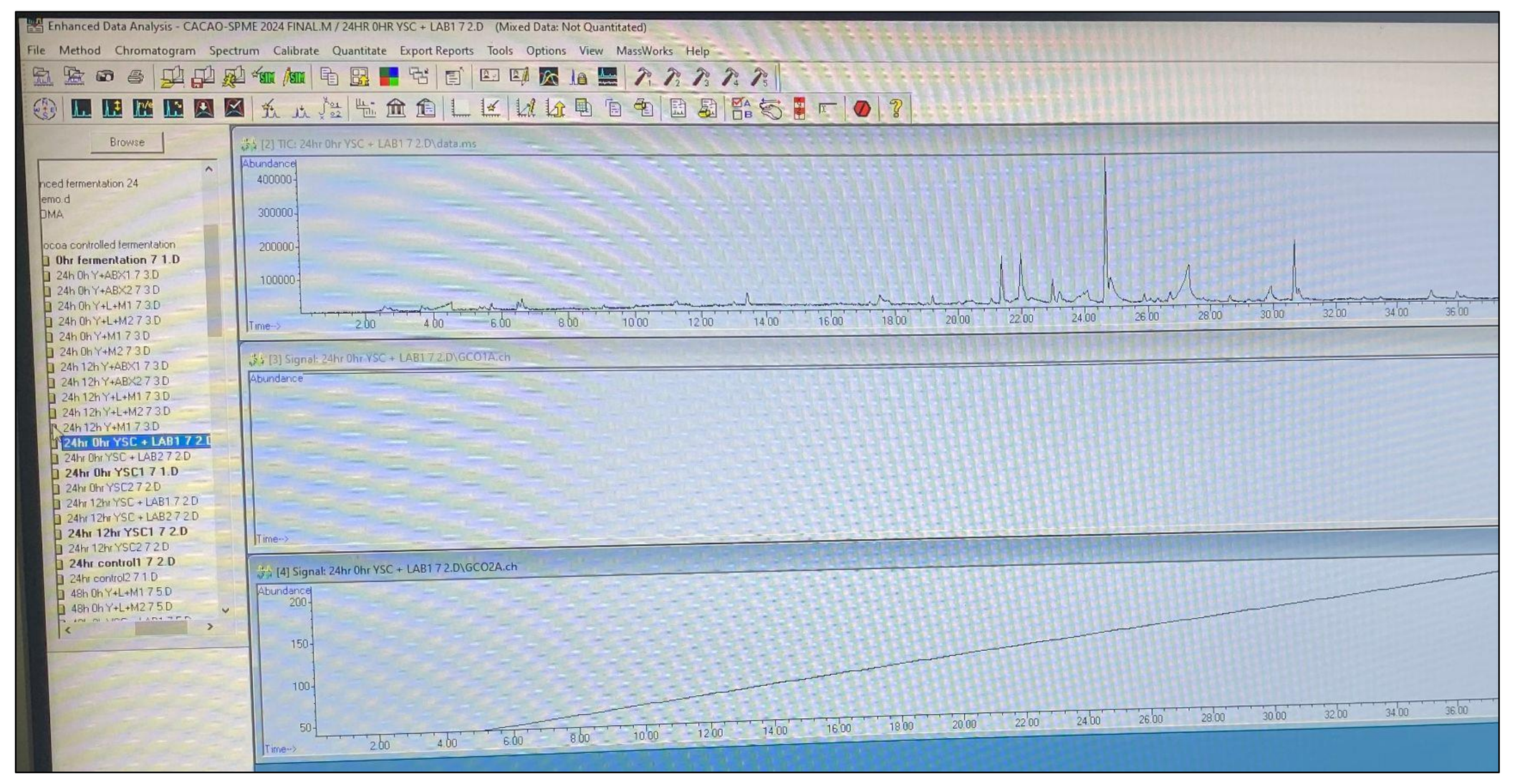
- Volatiles extracted via Solid Phase Microextraction - Gas Chromatography-Mass Spectrometry (SPME-GC-MS) from frozen beans and pulp
- Heated at 60℃, 20-min fiber exposure
- Analyzed for key aroma compounds



	Rate °C/min	Value °C	Hold Time min	Run Time min
(Initial)		40	3	3
Ramp 1	5	200	5	40

Post Run:	230 °C
Post Run Time:	5 min





METHODS

PCR and Sequencing (Microbial Analysis)

- DNA extracted at 48 hrs (PowerSoil Kit)
- PCR (Polymerase Chain Reaction) used to amplify 16S rRNA (for bacteria) and ITS regions (for fungi), targeting specific genetic markers for microbial identification
- High-throughput sequencing used to assess LAB & AAB diversity

Sequence
CAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAG
CAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAG
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CAGGCGGTTTTCTAAGTCTGATGTGAAAGCCTTCGGCTTAACCGGAGAAGTGCATCGGAAACTGGATAACTTGAGTGCAGAAGAGGGTAGTGGAACTCCATGTGTAG

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RESULTS

Volatile Compound Analysis (GC-MS):

High-throughput GC-MS revealed the presence of key desirable fermentation aroma compounds associated with high-quality cocoa, including:

•**Ethyl acetate** (fruity, sweet): Detected at high abundance (91.2% match in YSC 1 at 12h)
•**Octanoic acid, ethyl ester** (fruity, fatty): Consistently high abundance (81.4–87.1%) across early fermentation stages (0–12h)
•**Decanoic acid, ethyl ester** (sweet, waxy): Present up to 67.2%
•**2-Heptanol, acetate** (fruity): Detected in all samples, up to 50.8%
•**2-Pentanol, acetate** (fruity): Early indicator compound (61.6%)
•**Acetic acid, 2-phenylethyl ester** (floral): Moderate presence (23.5%, YSC 1 at 12h)

These compounds are widely recognized as positive fermentation markers due to their fruity and floral aroma contributions. Drug compounds (e.g., diclofenac, spironolactone) and siloxanes were excluded as contaminants or unrelated to fermentation.

Molecular Analysis (PCR & Sequencing):

DNA was extracted at 48 hours using the PowerSoil Kit, and PCR amplification of 16S rRNA and ITS regions was successful, as shown by gel electrophoresis. These regions were selected to target bacterial (LAB, AAB) and fungal diversity. All samples have since been successfully sequenced using high-throughput methods.

Each sample represents a unique combination of time point and starter culture condition, enabling analysis of microbial succession and diversity patterns across fermentation stages. Sequencing data will allow detailed comparison of community structure and the influence of experimental variables on microbial composition.

FUTURE STEPS

This project is part of a 10-week research program over multiple years, with several key steps remaining to complete the analysis and address the initial research objectives:

•**Sequencing Analysis:** Now that all PCR products have been successfully sequenced, microbial community composition (focusing on LAB and AAB populations) will be analyzed to assess diversity and succession across fermentation stages and starter culture conditions.
•**Aroma Profile Analysis:** Volatile compound data from GC-MS will be further analyzed using **Principal Component Analysis (PCA)** to visualize trends in aroma profiles across time points and treatments.
•**Integrated Microbial–Chemical Comparison:** PCA results will be compared to gene sequencing data to explore correlations between microbial diversity and the production of key aroma compounds. This integrative approach will help determine how microbial composition influences flavor development during cocoa fermentation.

These steps will help determine whether yeast starter cultures can be used to modulate microbial communities and enhance desirable aroma development in a controlled, repeatable way.