

Enzymatic Synthesis of Cocoa Butter Equivalent (CBE) using Oils and the Quantification of *cis* and *trans*

Isomeric Fatty Acids in Chocolate Products

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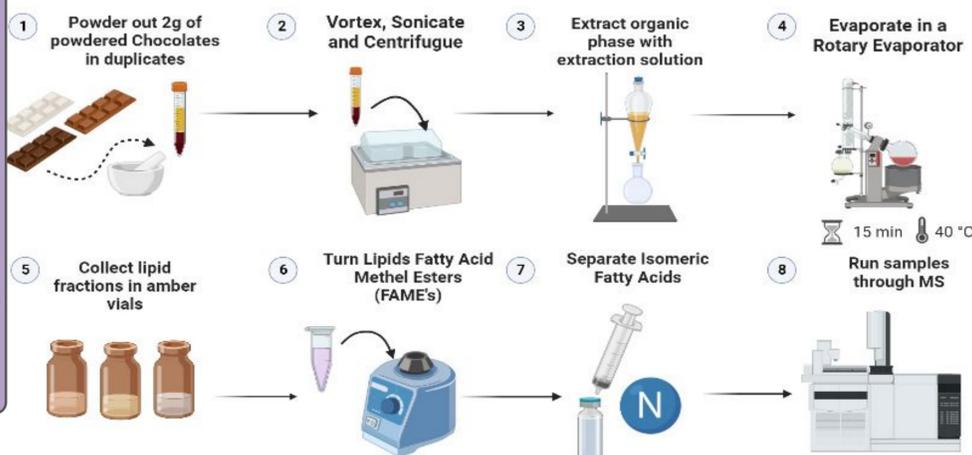
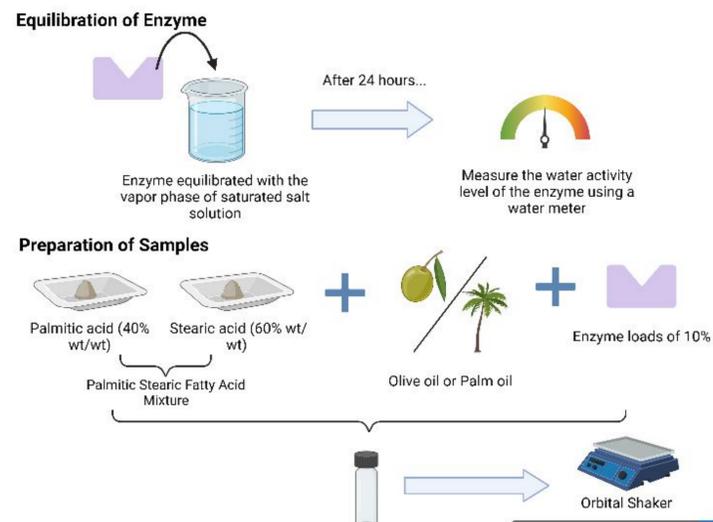
Introduction

- Cocoa butter equivalents (CBE) are derived from reconstructing of triacylglycerols (TAGs) within oils.
- As the demand for cocoa butter (CB) increases, the global supply decreases due to climatic conditions.
- In addition, in order to supply cocoa butter, there have been inhumane practices of child labor, therefore, resorting to synthesizing an equivalent assists in preventing such inhumanities.
- The main TAGs within olive oil and palm oil that will be prioritized during characterization:
 - POP, POS, POO, SOS, SOO
 - 1-palmitoy-2,3-dioleoyl glycerol is the target TAG
- Chocolates used for Quantitative study: milk, white, and dark (90% cocoa).

Objectives

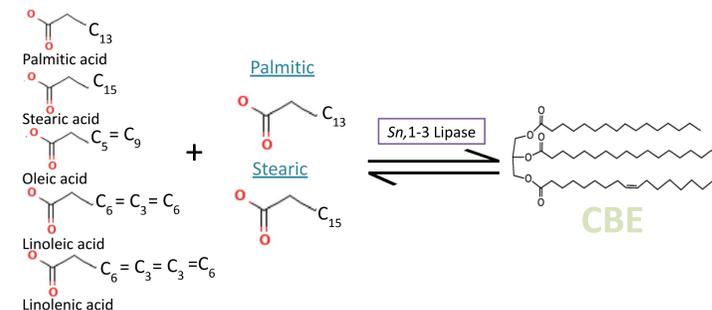
- Quantify fatty acids in chocolates
- Synthesize Cocoa Butter equivalents (CBE)
- Compare % peak areas of 1-palmitoy-2,3-dioleoyl glycerol (POO) over time

Procedures



Schematics

Synthesis of CBE



Quantitative Methyl Esterification of Chocolate Fats



Methods

Gas Chromatography Mass Spectrometry (GC-MS) and GC-Flame Ionization Detector for Quantitative Study

Column	FAMEWAX: 30 m, 0.25 mm x 0.25 μm (Restek)
Oven Temp.	200 °C (hold 1 min.) to 250 °C at 30°C/min (hold for 4 min.)
Detector Temp.	250 °C
Injector Temp.	250 °C
Injection Volume	1.0 μL
Carrier Gas	Helium
Split Ratio	1:100 (FID), 1:20 (MS)
Ion Source Temp. and Interface Temp.	Ion Source: 150 °C, Interface: 230 °C

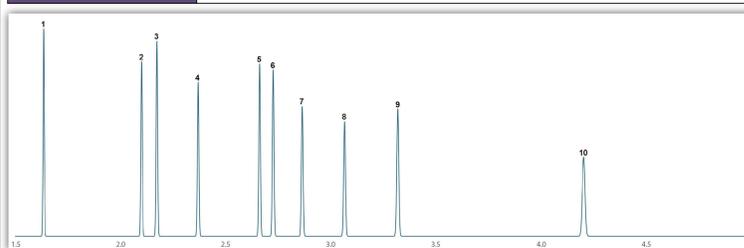


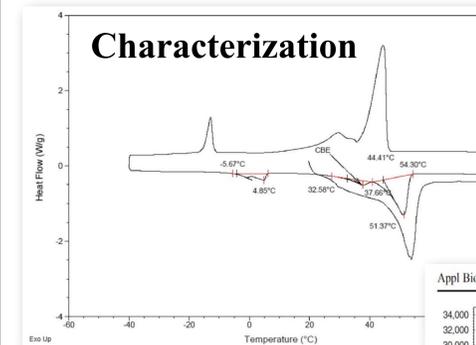
Figure 1. Predicted chromatogram of FAMES standard from Restek EZGC

1. C14:00 Methyl myristate (124-10-7), 0.1%
2. C16:00 Methyl palmitate (112-39-0), 26.8%
3. C16:01 Methyl palmitoleate (1120-25-8), 0.4%
4. C17:00 Methyl heptadecanoate (1731-92-6), 0.3%
5. C18:00 Methyl stearate (112-61-8), 33.7%
6. C18:01 Methyl oleate (112-62-9), 34.3%
7. C18:02 Methyl linoleate (112-63-0), 3.1%
8. C18:03 Methyl linolenate (301-00-8), 0.2%
9. C20:00 Methyl arachidate (1120-28-1), 1.3%
10. C22:00 Methyl behenate (929-77-1), 0.2%

References

- (1) Mohamed, I.O. *Appl Biochem Biotechnol.* **2015**, 175, 757–769.
- (2) Amorim, T. L. *et al. J. Agric. Food Chem.* **2019**, 67 (40),

Expected Outcomes



Characterization of Synthesis

DSC Polymorphic¹

Figure 2.

Appl Biochem Biotechnol (2012) 168:1405–1415

Kinetics

GC-MS

Peak composition %¹

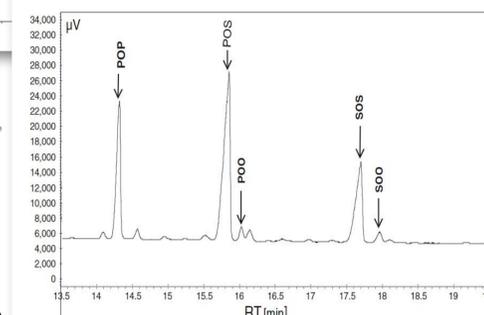


Figure 3.

- For the kinetic study, the triacylglycerol compositions will be measured over different time intervals.
- Percent peak areas will be used as an alternative to concentration.

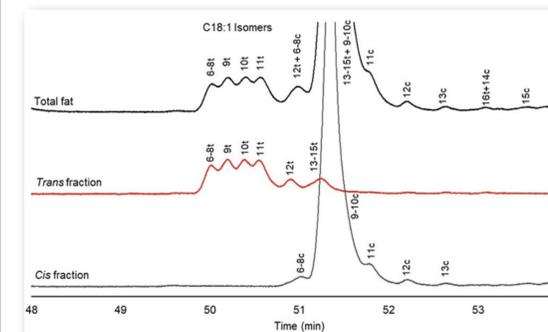


Figure 4.

GC-MS²

Future Works

- Run the FAMES samples and separated *cis/trans* FAMES on GC-MS.
- Begin synthesis of cocoa butter equivalent.

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