Time Dependent Selective Esterification of Free Fatty Acids in the Presence of Triglycerides

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Abstract

Background: There has been a shift over the years in the biofuel industry from food-based sources to nonedible ones such as algae. Algae can be grown to produce a high level of triglycerides (TAGs) that can be converted into biofuel. The algae biofuels industry is developing cost-effective ways to improve growth and harvesting techniques to convert the lipid fraction of the algae biomass into biofuels. In some cases, free fatty acids (FFAs) can also be produced during the treatment of harvested algae cultures. Both types of compounds, TAGs and FFAs, can be converted into biodiesel by using transesterification and esterification reactions of the TAG or FFA, Figure 1a and 1b respectively, with methanol in the presence of a catalyst to produce fatty acid methyl esters (FAMEs, aka: biodiesel). **Methods**: This work investigated and optimized the time dependence, and other reaction conditions, for selectively converting FFAs to FAMEs in the presence of TAGs reacted under the same conditions. Gas chromatography mass spectrometry (GCMS) was used for analyzing single and mixed-component samples of pure saturated and unsaturated FFAs and TAGs (Figure 2). **Results:** Complete conversion of FFAs to FAMEs was found to require less time and less rigorous conditions than TAGs and demonstrated the feasibility of using acid-catalyzed transesterification/ esterification reaction conditions to selectively determine the FFA content in the presence of TAGs. **Conclusions:** This research focused mainly on testing pure TAG and FFA compounds representative of those compounds commonly found in algae, but the time-dependent results also demonstrated the potential for modification of the analytical method typically used for algae biofuel and other bio-based samples.

Introduction/Research Question

Worldwide interest in algae as a potential source of renewable fuel has increased due to the potential economic and environmental benefits for replacing petroleum-based feedstocks. Existing industrial, large-scale methods utilize either base- or acid-catalyzed reactions to produce FAMEs from bio-based feedstocks, but these reactions result in the simultaneous and complete conversion of both FFAs and TAGs. Although base-catalyzed reactions are inexpensive and rapid to perform commercially, the sensitivity of the reaction to the presence of FFAs and water is a limitation. In the presence of a base, FFAs readily undergo saponification, resulting in the undesirable formation of soap that is difficult to clean up. Alternatively, acid-catalyzed reactions produce FAMEs from both FFAs and TAGs without unwanted byproducts. The draw-back to acid catalysts, however, is significantly slower reaction rates (i.e., increased reaction time) compared to base catalysts.

The total TAG content in algae cultures varies with nutrient availability during the growth of algae and is an important factor in determining the economic feasibility of algae as a feedstock for fuels, whereas the FFA content varies based on different extraction and treatment processes of TAGs extracted from harvested algae cultures and is important for determining the cost-effectiveness of large-scale treatment techniques. As with industrial-scale methods, current analytical sample preparation methods, such as the one used in our laboratory, are not capable of selectively distinguishing between FAMEs produced from FFAs or TAGs. ***This research involved the development of a modified sample preparation method, followed by GCMS analysis, that would allow for selective conversion of FFAs in the presence of TAGs.***

Methods

<u>Transesterification/Esterification Reaction Conditions</u>:

- Time: 5 – 120 minutes
- Temperature: 5 – 100°C
- Acid Catalyst: 4% H_2SO_4 in Methanol
- Compounds Tested: Pure FFAs (Figure 2)
  Pure TAGs
  FFA/TAG mixtures
  Certified mixture of 22 FAMEs

<u>Gas Chromatography Mass Spectrometry Analysis (GCMS)</u>:

- FAME Compound Identification by Mass Spectrometry
- FAME Quantification by using Internal Standard Compounds (Fig. 3)

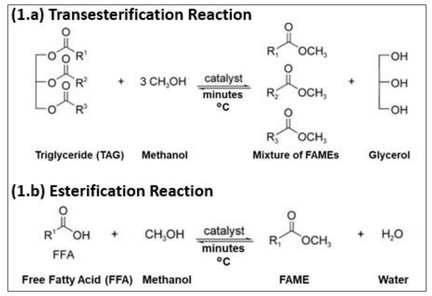


Figure 1. General Reaction Mechanisms of Producing Fatty Acid Methyl Esters (FAMEs) from Triglycerides and Free Fatty Acids. (1.a) Conversion of a carboxylic acid ester (TAG) to another ester (FAMEs) and glycerol. (1.b) Conversion of FFA to produce a FAME and water.

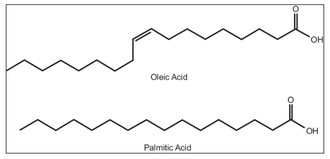


Figure 2. Chemical Structures of Common Long-Chain Fatty Acids: Palmitic Acid (C16:0) and Oleic Acid (C18:1). Palmitic acid is a sixteen-carbon saturated fatty acid chain with no "kinks" whereas oleic acid, which is an eighteen-carbon unsaturated fatty acid chain, depicts a "kink" or bend in the chain around the double bond.

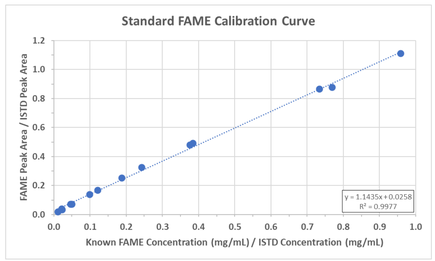


Figure 3. FAME Calibration Curve using an Internal Standard. Linear calibration plot of concentration ratio (known FAME / C19:0 ISTD) versus GCMS peak area response ratio (FAME PA / C19:0 ISTD PA) used for calculating the percent of conversion of the initial amount of FFA and TAG to FAME. Error bars are smaller than the symbol size.

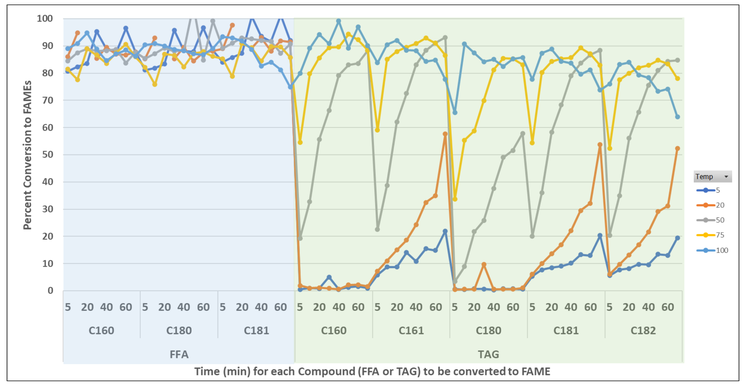


Figure 4. Time Dependent Conversion of Common Free Fatty Acids (FFAs) and Triglycerides (TAGs) Found in Algae Samples. Multiple FFA (blue shading) and TAG (green shading) compounds were reacted at different time, temperature, and other reaction conditions. The list of FFA compounds included palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1) and the TAGs were tripalmitin (C16:0), tripalmitolein (C16:1), tristearin (C18:0), trolein (C18:1), and trilinolein (C18:2). Complete conversion of FFAs to FAMEs occurred rapidly within 5 minutes at all temperatures, whereas the same reaction conditions for TAGs resulted in incomplete conversion of saturated TAGs and partial conversion of unsaturated TAGs to FAMEs at 5 minutes and low temperature (5 and 20°C). At higher temperatures (i.e., 50, 75, and 100 °C), the rate of conversion of saturated and unsaturated TAG to FAMEs was strongly dependent on reaction time, as indicated by the gradual increases in percent conversion observed for reaction times between 5 and 60 minutes.

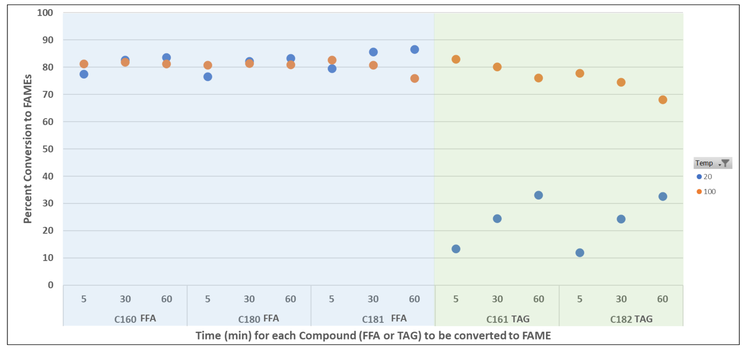
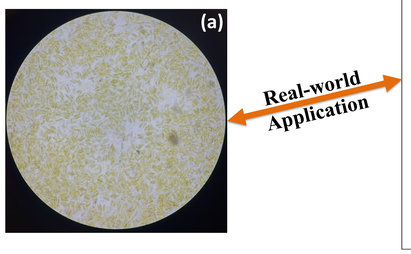


Figure 5. Verification of Time Dependent Conversion of a Mixture of FFAs and TAGs to FAMEs. Selective reactivity was successfully demonstrated by performing the reaction on a mixture of pure FFAs and pure TAGs followed by GCMS analysis. In this mixture, FFAs were identified by the presence of C16:0, C18:0, and C18:1 FAMEs while TAGs were identified exclusively by the presence of C16:1 and C18:2 FAMEs. The trends were consistent with results shown in Figure 4.



Real-world Application

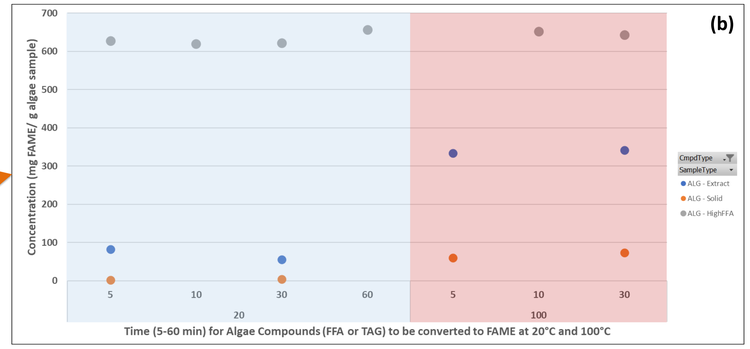


Figure 6. (a) Image of ~250 whole lyophilized algae cells (GAI sample #33789) viewed from a compound optical microscope with 400x total magnification. **(b)** Application of time and temperature dependent reaction conditions to the conversion of FFAs and TAGs (to FAMEs) found in three different real-world algae sample types: (●) lyophilized algae (primarily TAGs), (●) algae extract sample (mixture of FFAs and TAGs), and (●) algae extract sample (high FFA content). Results were consistent with prior experiments where cooler reaction temperature (blue shading) and shorter reaction times resulted in the complete conversion of FFAs and partial conversion of TAGs. Both FFAs and TAGs were completely converted at higher temperature (red shading) and longer reaction time.

Results and Conclusion

- A set of reaction conditions aimed at the selective conversion and determination of FFAs in the presence of TAGs, using GCMS for analysis, identification, and quantitation of FAMEs, was evaluated.
- Time dependent, selective determination of FFAs in the presence of TAGs was successfully demonstrated in laboratory prepared samples (Figures 4 and 5) and real-world algae samples (Figure 6).
- Complete conversion of C16:0, C18:0, and C18:1 FFAs at short reaction time and room temperature (Fig. 4, 20°C) was observed whereas the same reaction conditions resulted in incomplete conversion of saturated C16:0 and C18:0 TAGs and partial conversion of unsaturated C16:1, C18:1, and C18:2 TAGs to FAMEs.
- At higher temperatures, conversion rates of both saturated and unsaturated TAGs to FAMEs was strongly dependent on reaction time, as depicted by the gradual increases in percent conversion observed between 5 and 60 minutes.
- In a laboratory-prepared mixture, FFAs were identified by the presence of C16:0, C18:0, and C18:1 FAMEs while TAGs were identified exclusively by the presence of C16:1 and C18:2 FAMEs. The trends observed for conversion of saturated and unsaturated compounds in Figure 5 were consistent with results obtained for individual compounds in Figure 4.
- Application of the reaction conditions to the conversion of FFAs and TAGs (to FAMEs) found in three different real-world algae sample types appeared to be consistent with previous test results obtained from individual compounds and FFA/TAG mixtures.
- ***Reactivity conditions for FFAs and TAGs were found to be different, thus there is strong potential to use time dependent esterification reactions to distinguish the FFA content from the TAG content in the GCMS analysis of complex bio-based samples.***

Future Work

1. Determine why our conversion percentages were not 100%.
2. Determine if TAG or FAME degradation was occurring for longer reaction times and higher temperatures for TAGs compounds.
3. Method validation needed to confirm preliminary experimental results.
4. Apply the test method against more customer algae samples.
5. Investigate the effect of different reactivity rates observed for unsaturated and saturated TAGs.

References and/or Acknowledgments

1. Kail, B.W.; Link, D.D.; & Morreale B.D. (2012). Determination of Free Fatty Acids and Triglycerides by Gas Chromatography Using Selective Esterification Reactions. *Journal of Chromatographic Science*, *50*(10), 934-939. https://doi.org/10.1093/chromsci/bms093.
2. Fall 2022 and Spring 2023 ***CHEM 495: Directed Research in Chemistry*** projects funded by the Biology & Chemistry Department
3. Real-world algae samples selected from archived fee-for-service samples from Global Algae Innovations (GAI)