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A Novel Method of Analyzing Free Fatty Acid Content in Triacylglycerol Mixtures using ^{1}H-NMR

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Abstract

Biodiesel has become a popular option as a potential renewable energy source because pure vegetable oils can be easily transesterified into methyl esters by using a base catalyst. Used vegetable oils (UVOs), such as cooking grease, may be an environmentally friendly source of biodiesel. However, UVOs are challenging to transesterify because the triacylglycerol molecules have hydrolyzed, resulting in the formation of free fatty acids (FFAs). These FFAs ultimately hinder formation of the desired methyl ester product. This problem can be alleviated by using a titration, which indirectly determines the quality of the oil by determining FFA content. While a titration is reliable, it can be time intensive and inaccurate. Proton NMR was investigated as a more reliable method to quantitate FFA content. By comparing the ratio of the proposed diacylglycerol moiety relative to the glycerol peak and determining the acid number via titration, a correlation was found. Thus, analyzing the diacylglycerol moiety relative to the glycerol peak could be a quick and effective way of determining the FFA content of an oil sample.

Keywords: Biodiesel, Acidity, ^{1}H-NMR

1. Introduction

Renewable energy sources are currently very popular due to their potential to reduce adverse environmental impacts produced by fossil fuels as well as their unlimited abundance and availability.[1] One particular renewable energy source that is gaining quick popularity is biodiesel; a recent poll released from the U.S. Energy Information Administration found that 1.34 billion gallons of biodiesel was produced in 2013, a number that has increased 150 fold since 2001.[2] Furthermore, a study performed by the Environmental Protection Agency demonstrated that biodiesel has the capability of producing less carbon monoxide and hydrocarbon emissions than petroleum.[3]

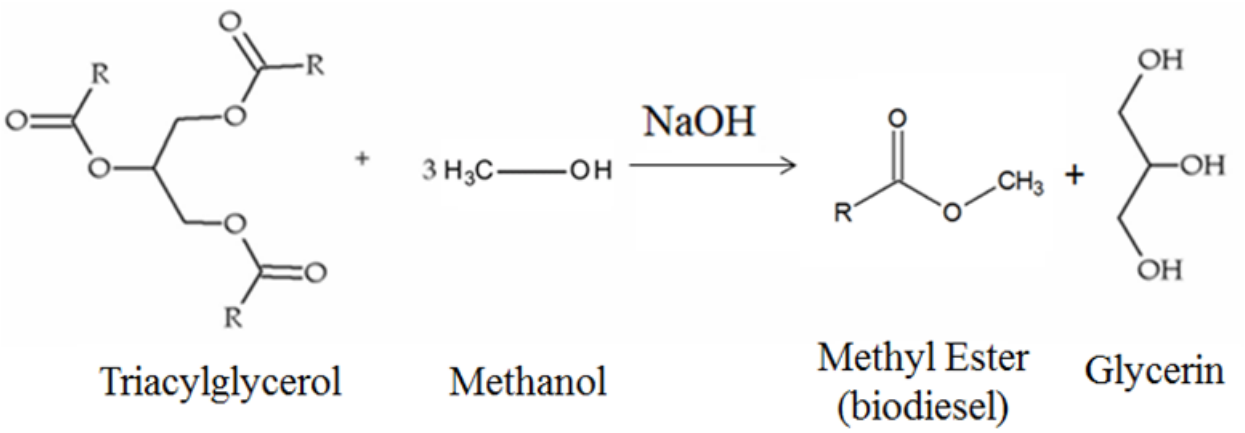

Figure 1: Base catalyzed transesterification reaction used to convert triacylglycerols extracted from plants into biodiesel

Much of the current research on biodiesel has been focused on its industrialization process. Generally triacylglycerols are extracted from crops (corn, soy, etc.). These triacylglycerols are then converted into biodiesel using a base-catalyzed transesterification reaction (Figure 1).[4] This process works quite well in biodiesel production. For example, it was shown this reaction can be optimized to obtain biodiesel production at 98% yield.[5] Furthermore, the workup is fairly straightforward as the transesterification reaction produces a biphasic layer where the biodiesel (top layer) can be easily isolated from the dense glycerol byproduct (bottom layer).

There has also been a recent push to extend the industrialization processes in order to recycle used vegetable oils (UVOs) such as cooking grease and fryer oils into biodiesel[6]. Although this does have economic and environmental potential, the chemistry becomes more complicated because UVOs have high amounts of free fatty acid (FFA) compared to pure vegetable oils commonly extracted from plants. The catalyst commonly used in base-catalyzed transesterification would react preferentially with the FFA to generate a soap. This soap formation is problematic because it poisons the base catalyst and the soap ultimately results in an emulsion that effectively eliminates biphasic separation of biodiesel and glycerol. This problem can be potentially bypassed by measuring the amount of FFA present in an oil sample, thereby determining whether or not the oil sample can be effectively converted into biodiesel.[7]

The standard method for FFA quantification is an acid-base titration used to determine the acid number (expressed in mg KOH/g fat). Although this method is reliable for pure oils, it can be timely and environmentally unfriendly. It can also be inaccurate when titrating darker oils because it is more difficult to distinguish the precise endpoint denoted by a pH indicator. Thus, a lot of research has focused on replacing this method with modernized instrumental techniques. These techniques have the potential to be useful in determining the FFA content of oils with a higher accuracy and a greater throughput. Some of these instrumental techniques have already been explored: methods have been determined to quantitate FFA content using ^{13}C NMR and IR spectroscopy that have bypassed some of the shortcomings of the titration method mentioned above.[8, 9]

Here, we present our findings on a quick and novel method that could be useful in determining the FFA content in oils using ^{1}H NMR. We analyzed the integration value of a particular diacylglycerol moiety in different oil compositions of used and pure vegetable oils, which was compared to the standard titration method. Our results suggest that our method has the potential to be used to quantitate FFA content in oils.

2. Methods

2.1 Materials

Crisco brand olive oil was acquired commercially while two of the following compositions of high acid number oils were obtained from D.A. Stewart: 70% Yellow grease, 30% Century MO5 (YG1) and 65% Yellow grease, 35% Century MO5 (YG2). The following compounds were obtained from Sigma-Aldrich: 99.5% Toluene, 97% anhydrous sodium hydroxide pellets and phenolphthalein pH indicator, while 99% 2-propanol was obtained from Fisher Scientific.

2.2 Composition of used oil and fryer oil samples

Olive oil was mixed in the following volumetric ratios with either YG1 or YG2: 3:1, 2:1, 1:1, 1:2, and 1:3 and was then subjected to ^{1}H NMR analysis and an acid-base titration. The same protocol was followed with pure samples of olive oil, vegetable oil, vegetable + canola oil, YG1, and YG2. Oils with high FFA compositions were also generated by imitating fryer conditions based on information reported previously. [6] Aliquots of 40 mL olive oil were placed in four 1L beakers and the following components were added to each beaker: **1.** no additional components, **2.** 300 mL H_2O, **3.** 300 mL H_2O + 1 pellet NaOH (~9*mM*), **4.** 300 mL H_2O + 2 NaOH pellets (~18*mM*). Each beaker was incubated at 177^{o}C for five days. Burnt fat was isolated for each beaker by soaking them in toluene for 3 days followed by decantation. The toluene mixtures were heated at 50^{o}C for five days to evaporate off toluene and minimize splatter, after which they were allocated for titration and ^{1}H NMR.

2.3 Acid-base titration

An acid-base titration was used to quantify the amount of FFA present by analyzing the acid number of each oil sample. Phenolphthalein solution (1% by volume) was added to a 1:10 parts fat sample to 2-propanol mixture. A 0.01M Sodium Hydroxide (NaOH) titrant was added to the fat mixture until the mixture changed to a dark purple color. The volume of NaOH added to achieve this color change was used to determine the acid number of the corresponding fat sample.

2.4 ^{1}H NMR acquisition

Two drops of each oil sample were placed in an NMR tube followed by addition of 0.7mL $CDCl_3$. ^{1}H NMR data was obtained using a 300 MHz magnet and the Bruker TopSpin 1.2 program. Acquisition parameters of ^{1}H NMR spectra included a 20 second relaxation delay with 32 scans and 2 dummy scans for the optimal accuracy of integration values.

2.5 Quantification of FFA content

The proposed NMR method involved quantifying the "diacylglycerol bump" (DAG bump) integration value located on the right glycerol peak (Figure 2). This was done by taking a ratio of the DAG bump integration value to the integration value for the remainder of the glycerol peak (4.2-4.4 ppm). Each of the DAG bump integrations obtained from NMR spectra were correlated to their corresponding titration value.

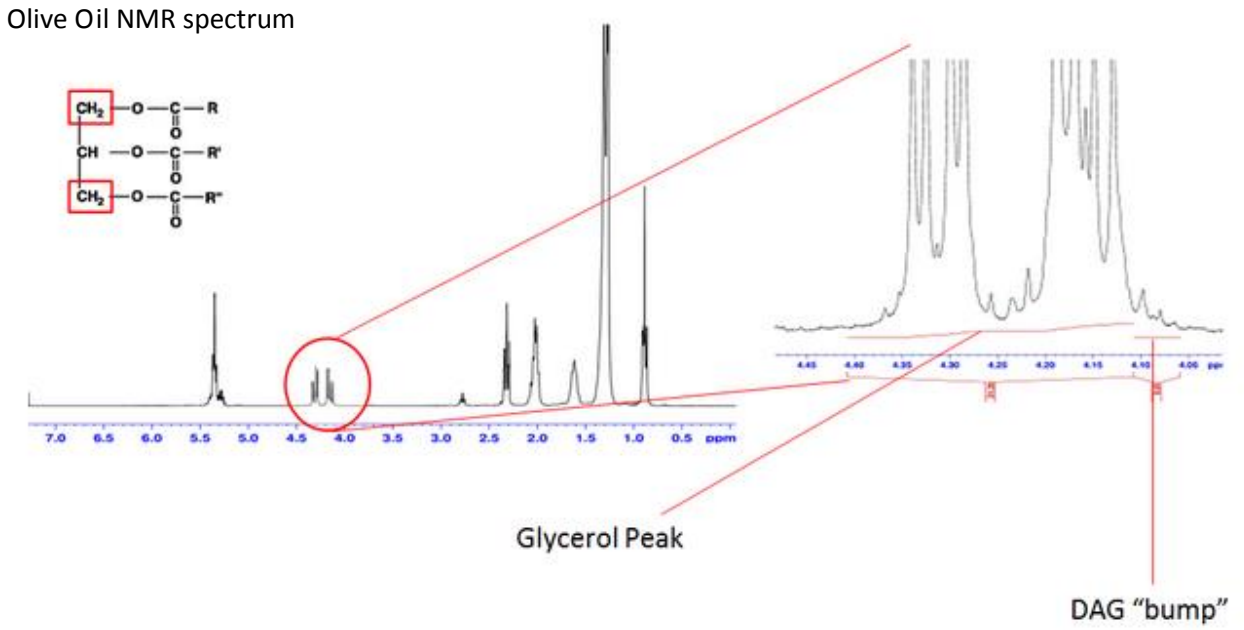

Figure 2: ^{1}H-NMR of olive oil with emphasis on the glycerol peak (4.2-4.4ppm), outlining DAG bump quantification

3. Results and Discussion

Previous data suggests that the ^{1}H NMR moiety corresponding to the -CH_2 group on the glycerol backbone shifts up-field as a TAG is hydrolyzed into a DAG.[10] Thus, this up-field shift could result in the presence of a distinct moiety located on the glycerol peak, confirmation of this observation allowed us to deem this putative moiety "the DAG bump" (Figure 2). In order to determine whether our proposed DAG bump was viable for quantifying FFA content in oils, we made different compositions of oils with different amounts of FFA content. The DAG bump ratio obtained via ^{1}H NMR was correlated with the titration method that is used commonly in industry for each oil sample (Figure 3A). Our results suggested that a direct correlation existed between the DAG bump and the acid number, implying this technique could be viable in determining the FFA content in an oil mixture.

A study correlating FFA content and the quality of oil suggests that oils with an acid number of approximately 10mg KOH/g fat or lower could be converted to biodiesel in high yield after pre-treatment to reduce FFA content.[11] This suggests that oils with these acid numbers could be valuable to convert to biodiesel on an industrial scale. Consequently, we analyzed the viability of our proposed method on acid numbers lower (Figure 3A.1) and above (Figure 3A.2) an acid number of 10mg KOH/g fat. We found a positive correlation between the acid number and the DAG bump ratio for oils with low and high FFA content. However, this correlation was much stronger in oils with a higher acid number than in oils with a lower acid number as evidenced by the greater correlation of determination (Figures 3A.1 and 3A.2). This discrepancy may have been due to the concentration of the base titrant used to titrate pure vegetable oils (the difference between the acid number of olive oil and vegetable oil was a matter of 2-3 drops). Diluting the titrant in future experiments could yield more accurate acid numbers for pure vegetable oils and mitigate the discrepancy observed between oils with high and low FFA content.

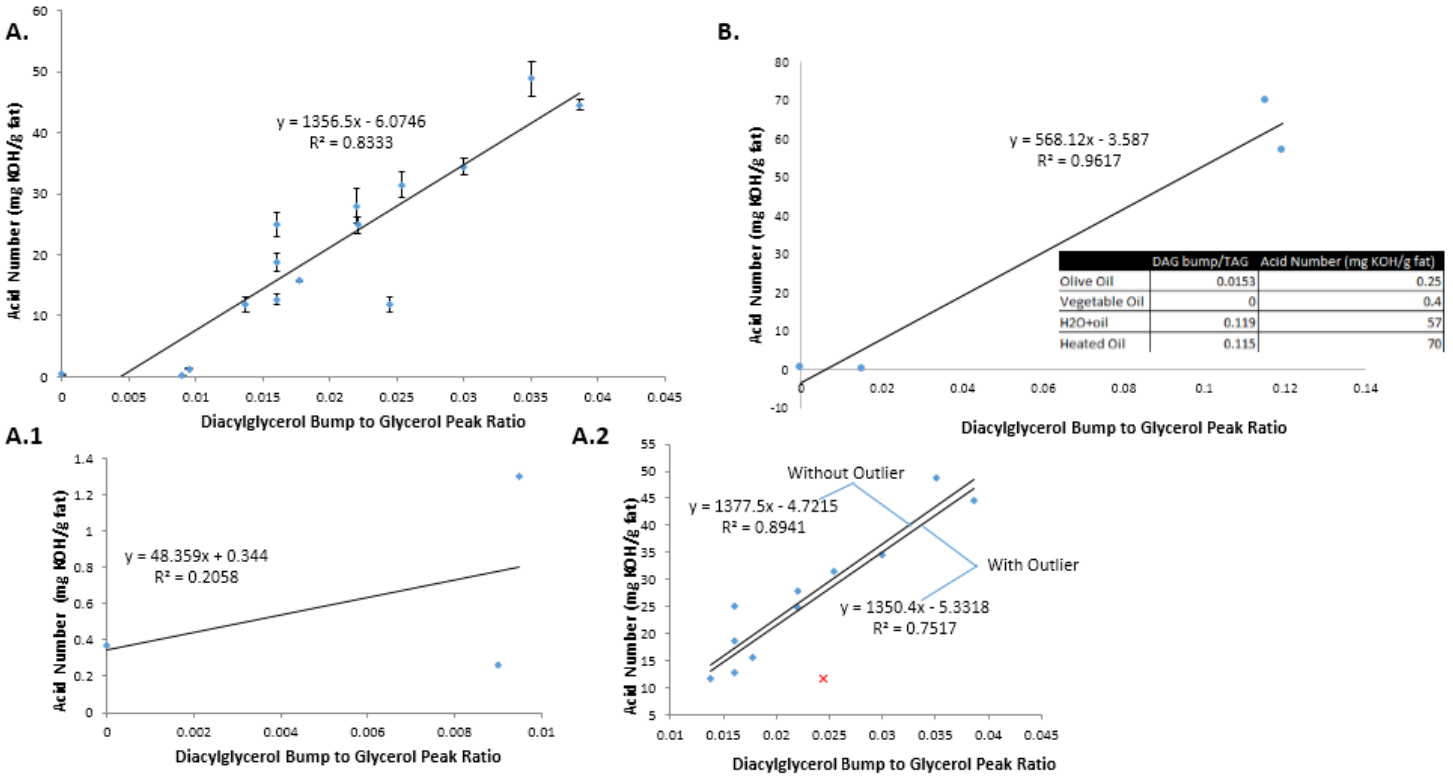

Figure 3: A. Correlation between the DAG bump ratio and the acid number of all oil compositions. A.1 Correlation shown for oils with low FFA content acid number (<10mg/g fat). A.2 Correlation specifically shown for oils with high FFA content (acid number >10mg KOH/g fat), the red X denotes the outlier that was excluded in the best fit line labeled "without outlier." B. Correlation shown for fryer simulated oils with vegetable oil and olive oil as negative controls.

Although a correlation between the DAG bump and FFA content appears evident, the fryer conditions necessary for UVO formation could hinder this correlation. For example TAG oxidation and polymerization side reactions can compete with hydrolysis which would add additional variables into the correlation.[12] Thus, we also analyzed whether the same correlation between the DAG bump and FFA content would be evident by taking olive oil and subjecting it to different conditions that are commonly found within a fryer. The positive correlation seen between the DAG bump ratio and the acid number was still present between the fryer-treated olive oil compared to the untreated olive oil and vegetable oil control (Figure 3B). However, it is important to note that the similar DAG bump ratios in the fryer treated samples with and without water corresponded to considerably different acid numbers (Figure 3B). This discrepancy could have resulted from potential error between the calculated acid number for the different fryer simulated oils. For example, we were only able to isolate 8-10 mg in 2 of the 4 original samples because we were unable to effectively re-suspend the oil that adhered to the sides of the beaker. Consequently, we could only perform two titrations of each of our fryer treated oils by using a 50 fold diluted base titrant. Furthermore, the dark brown color of the fryer treated oils made the detection of the exact titration endpoint problematic. Therefore, multiple trials are most likely necessary to determine this endpoint more distinctly. Future experiments will be performed to optimize this technique in order to generate more precise results with a greater sample size.

Ideally, if the DAG bump has a direct correlation with the acid number, then we would expect the slopes relating these two parameters in the high acid number oils and the fryer simulated oils to be equal. However, the high acid number and fryer simulated oils possessed markedly different slopes (Figures 3A+3B), suggesting that the relationship between the acid number and the DAG ratio differs between the high acid number and the fryer-

simulated oils. This information suggests that a standard curve may be needed for this method to be accurate. This would not be ideal, as running a standard curve for each sample would be time intensive (thus eliminating the purpose of using this protocol over other instrumental techniques used for FFA quantification). However, as addressed above, some of the inaccuracies generated in the fryer method protocol could change the value of the slope dramatically.

The fryer simulation method may have not been a sufficient representation of fryer conditions. For example, oils in a deep fryer are exposed to food a multitude of times before the FFA content increases. This is because frying food in heated oil results in an influx of water and oxygen that initiates the breakdown of the fatty acids on the glycerol backbone[12]. As a result, the FFA content of frying oil increases with the number of uses.[13] Due to relatively lower water and oxygen levels, our fryer simulation may have led to a lower FFA content in our samples than what would typically be seen in frying oils. In order to remedy this, future experimentation will involve gathering fryer oil samples from restaurants and determining the acid number and DAG ratio for each sample. This experiment would allow us to better determine the practicality of our ^{1}H NMR technique in evaluating the quality of fryer oil from commercial businesses that could be used for biodiesel production.

4. Conclusions

Despite the setbacks evident in our fryer simulated experiment, the strong correlation between the DAG bump and the acid number shows promise for the proposed FFA quantification method using proton NMR. Although this novel technique is not ready to be applied for industrial purposes, it certainly warrants further investigation. Our results suggest that the DAG bump could signify whether a large lot of oil can be feasibly converted into biodiesel (i.e., if it has an acid number above or below 10mg KOH/g fat). However, the discrepancies between the high acid number and fryer simulated oils would need to be mitigated before the DAG bump method can be used to determine the FFA content more precisely. Although others have determined different ^{1}H NMR techniques to evaluate FFA content,[14,15] our method is particularly useful because of its speed and convenience. Only the glycerol moiety needs to be integrated and no reference peak is necessary. After the glycerol peak is integrated, the DAG bump is simply separated and acts as the numerator whereas the remaining glycerol peak acts as the denominator in the ratio. Furthermore, this technique can be performed in deuterated chloroform whereas others have proposed methods using a more complex solvent system; [16] although this alternative method was effective, it is also less convenient and more costly.

5. Acknowledgements

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