

Gas Chromatographic Determination of Glycerol and Triglycerides in Biodiesel from Jatropha and Castor Vegetable Oils

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Abstract. Monoacylglycerols and diacylglycerols are intermediate compounds in biodiesel which result from incomplete transesterification reaction during biodiesel production. Traces of free glycerine and partially reacted triacylglycerols are also found in biodiesel. These contaminants cause serious operational problems in engines, such as engine deposits, filter plugging, and emissions of hazardous gasses. Increased levels of these contaminants in biodiesel compromise quality which is vital for commercialisation of this product. In this work, levels of free glycerine and total glycerine in jatropha methyl ester (JME) and castor methyl ester (CME) were determined using gas chromatography (GC) equipment. Amounts of free and total glycerine in JME and CME were generally high compared to the ASTM D6751 and EN14214 recommended values. Free glycerine from JME was 0.1% wt compared to 0.02% wt (ASTM D6751) and 0.01% wt (EN14214) values whereas the total glycerine from JME was 2.96% wt compared to 0.24 %wt (ASTM D6751) and 0.21% wt (EN14214). These discrepancies could have resulted from insufficient purification of the product and incomplete conversion or due to the high temperature associated with GC analysis that might have caused pyrolysis or thermal degradation of certain lipid components. Castor methyl ester free glycerine was 0.14% wt while total glycerine was 13.21% wt. This can still be explained by the same reasons given for JME. Thermal decomposition of lipid components in a GC could have interfered with the summative mass closure calculations that were done to determine the total composition of the biomass.

Introduction

Biodiesel is a renewable and biodegradable fuel refined from vegetable oils and animal fats. It has captured world attention as an alternative diesel fuel for use in compression ignition (CI) engines [1]. Biodiesel has superior properties than petroleum diesel and has better lubricating ability desirable for moving parts of an engine [2,3]. Biodiesel technology therefore has expanded from a research effort to a commercial enterprise [4]. Technologies for biodiesel production vary but not limited to, direct use and blending of oils, micro-emulsions, pyrolysis and transesterification [2,4].

Transesterification is the most preferred method for commercial production of biodiesel because it is faster and less expensive than the other methods. It is a reaction of a lipid with an alcohol to form esters and glycerol. In principle, it is the action of an alcohol displacing another from an ester, also known as alcoholysis (cleavage by an alcohol) [5,6,7,8,9].

Transesterification can be base catalysed, acid catalysed, enzyme catalysed or non catalysed [7]. Excess alcohol is used to shift the reaction towards the product formation since the reaction is reversible. For this, primary monohydric alcohols (C1 to C8) are used, for example, methanol,

ethanol, propanol, butanol and Amyl alcohol [4]. Methanol has been the most preferred alcohol as it is less expensive and has the shortest chain that gives some favourable properties like quick reaction with triglycerides and easy dissolution of sodium hydroxide catalyst.

During transesterification, monoacylglycerols and diacylglycerols are formed as intermediate organic compounds. Small amounts of these compounds remain in biodiesel product as contaminants. Un-removed glycerols, partially reacted triacylglycerols, free fatty acids (FFA), residual alcohol and residual catalyst can contaminate the final product as well [1,10,11,12]. Incomplete transesterification and insufficient purification of the fuel product can lead to serious operational problems in the engine such as engine deposits, filter plugging and eventually hazardous emissions result [1,13,14,15].

It is also known that every vegetable oil has its own characteristic fatty acids and since biodiesel is produced from different vegetable oils of varying origins and qualities, it is necessary to standardize the fuel quality in order to guarantee engine performance without problems [12,15]. Biodiesel quality determination is therefore vital for its successful commercialization. Foglia *et al.*, [16] reported that regardless of the feedstock used for the biodiesel production or the transesterification process carried out, it is necessary to determine the degree of purity and the type of contaminants present in the biodiesel before its use. Currently, the EN14214 is the standard in use all over Europe and EN14105 deals with the determination of free and total glycerol in methyl esters (biodiesel). In the United State of America, the American Society for Testing and Materials (ASTM D 6751) standard exist for determining the quality of biodiesel and blends and ASTM D 6584 deals with free and total glyceride contents of biodiesel.

In all the above mentioned standards, restrictions are placed on contaminants such as free and total glycerine for limiting glycerols and acylglycerols; flash point for limiting residual alcohol; acid value for limiting FFA and ash value for limiting residual catalyst [12]. Methods have been developed to monitor these contaminants in biodiesel fuel. Examples of existing methods include Fourier Transform Near Infra Red (FT-NIR), High Performance Liquid Chromatography (HPLC), Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy (31P NMR) and Gas Chromatography (GC). GC method has widespread use due to easy adaptability, high precision and accuracy and moderate costs compared to the other methods mentioned above [17]. However, the high temperatures associated with GC can cause pyrolysis and thermal degradation of certain lipid components. This can interfere with the summative mass closure calculations that are typically done to determine the total composition of biomass [14]. In this work, ASTM D 6584 and EN14105 methods were used to determine concentrations of free and total glycerine contaminants in biodiesel produced from *Jatropha curcas* and castor beans oils using Gas Chromatography (GC). The following biodiesel properties were already determined in our earlier work; flash point, cloud point, density, viscosity, acid value and heating values [18].

Materials and Methods

Materials: Reference standard reagents used were solutions of glycerine, 500 mg/l; monoolein, 5,000 mg/l; 1,3-diolein, 5,000 mg/l; and triolein, 5,000 mg/l. Internal standards used were 1,2,3-butanetriol, 1,000 mg/l; tricaprin, 8,000 mg/l, and N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA), all in pyridine solvent. They were obtained from Choice Analytical Pty Ltd., Thornleigh NSW 2120 Australia. Normal hexane (n-hexane), 99% min assay was obtained locally from Kampala. *Jatropha* and castor seeds for preparation of fatty acid methyl esters were obtained from Arusha and Dodoma regions, respectively in Tanzania.

Sample and Standards Preparations: *Jatropha* Methyl Ester (JME) and Castor Methyl Ester (CME) were prepared according to the methods described by Freedman *et al.* [19] and Plank and Lorbeer [20]. Standards and sample preparations were done according to ASTM D 6584 and EN

14015 standard procedures using solutions of glycerol, monoolein, diolein, triolein, 1,2,3-butanetriol and tricaprln. Five different levels of concentrations were made as indicated in Table 1. Standards were weighed into five 10 ml vials as indicated and then derivatized by adding 100 μ L of MSTFA solution to each vial and kept for 15 to 20 minutes before adding n-hexane to top up the vials. Standards (1 μ L) were injected onto AOC-20i split-less port of the GC for analysis.

Table 1: Weights of standard solutions used

Standards	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5
Glycerol [mg]	2.2	21.4	45.1	62.5	71.2
1,2,3- Butanetriol [mg]	95.1	95.3	100.6	101	85.8
Monolein [mg]	16.3	47.8	96.9	144.3	194.3
Diolein [mg]	7.8	5.8	38.6	66.8	95.9
Triolein [mg]	9.7	19.1	38.3	66.4	93.8
Tricaprin [mg]	95.7	62.3	95.2	95.9	94.1
MSTFA [mg]	97	97	97	97	97

Samples were weighed (50 mg) into 10 ml vials. These were derivatized in the same way like the standards by adding 100 μ L of MSTFA solution and waiting for 15 to 20 minutes then n-hexane was used to top up the vial. Samples (1 μ L) were injected onto an AOC-20i split-less port for analysis. Derivatization was necessary here to improve the volatility of the hydroxyl groups of the triglycerides and make good peak shapes.

Experimental Procedure: Analysis of samples was performed using a Shimadzu 2010 GC-17AAF V3, Japan. The instrument was equipped with FID detector and Auto sampler AOC-20s with auto injector AOC-20i. The column was a non-polar Rtx- biodiesel (TG); 10 m x 0.32 mm ID x 0.1 μ m fitted with a column guard 1 m x 0.53 mm ID by leak free high T purged connection. A sample (1 μ L) was injected onto a split-less column set at 50°C. After one minute, the GC oven was raised to 180°C at 15°C/min. It was then increased to 230°C at 7°C/min. Finally, it was increased to 380°C at 30°C/min and held there for 10 minutes. The resulting chromatogram peaks were driven off the column using hydrogen carrier gas at 3 mL/min and detected using an FID.

Reference standards were used for peak identification by comparison of the sample peaks with reference standard peaks. Peak separations were based on carbon numbers; mono, di and triglycerides were separated according to carbon numbers. Glycerides eluted before the methyl esters, followed by monoglyceride, then diglyceride and finally triglycerides. The calibration curve was calculated using equation (1)

$$W_s/W_{is} = a \cdot (A_s/A_{is}) + b \quad (1)$$

Where: W_s = weight of reference component (mg); W_{is} = weight of internal standard (mg); a = slope of calibration function; b = intercept of calibration function; A_s = peak areas of reference component and A_{is} = peak area of internal standard.

For quantitative determinations, glycerine was quantified using the calibration function based on Internal Standard 1,2,3-butanetriol (IS1). Whereas mono-, di- and triglycerides were calculated according to the calibration function based on the Internal Standard Tricaprin (IS2). Individual peaks for monoglyceride were integrated separately and the total concentration was obtained by summing up the concentrations of the individual monoglyceride. The same procedure was also applied for diglyceride and triglycerides. It is important to note that it was not possible to produce clear peaks of glycerine and 1,2,3-butanetriol due to masking and superimposition of the peak glycerine.

The temperature programme was made in such a way as to separate classes of compounds like monoglyceride, diglyceride and triglycerides respectively. After sample peak identifications, the mass percentages of glyceride, monoglyceride, diglyceride and triglycerides were determined using the slopes and intercepts obtained from the calibration functions as given in the equations (2) and (3).

$$\text{Glycerine: } G = (a_g \times A_g/A_{is1} + b_g) \times W_{is1}/W \times 100 \quad (2)$$

Where: G = weight percent of glycerine in the sample; a_g = slope of glycerine calibration function; A_g = peak area of glycerine; A_{is1} = peak area of internal standard 1; b_g = intercept of the calibration function for glycerine; W_{is1} = weight of internal standard 1 (Butanetriol) (mg); W = weight of sample (mg).

$$\text{Individual glycerides: } Gli = (a_{01} \times A_{gli}/A_{is2} + b_{01}) \times W_{is2}/W \times 100 \quad (3)$$

Where: Gli = weight percent of individual glycerides in the sample; a_{01} = slope of the calibration function for mono-, di-, or triolein; A_{gli} = peak area of individual glycerides; A_{is2} = peak area of internal standard 2; b_{01} = intercept of calibration function for mono-, di-, or triolein; W_{is2} = weight of internal standard 2 (Tricaprin) (mg); W = weight of sample (mg).

Results and Discussion

Figure 1 shows the chromatograms of standard reagents and Figure 2 shows those of glycerine and 1,2,3-butanetriol. These are typical of the chromatograms for biodiesel standards that are normally reported in literature [21,22] meaning that the equipment was well calibrated.

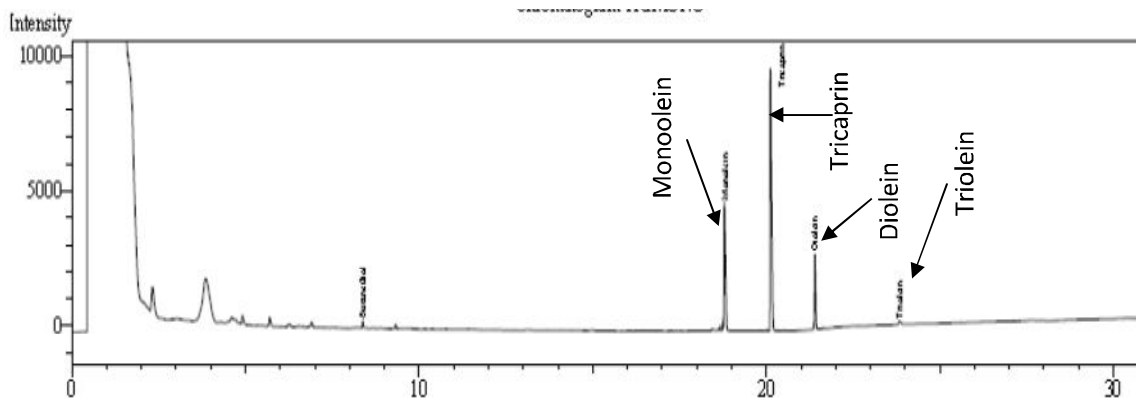


Figure 1 Chromatogram of monoolein, diolein and triolein standards

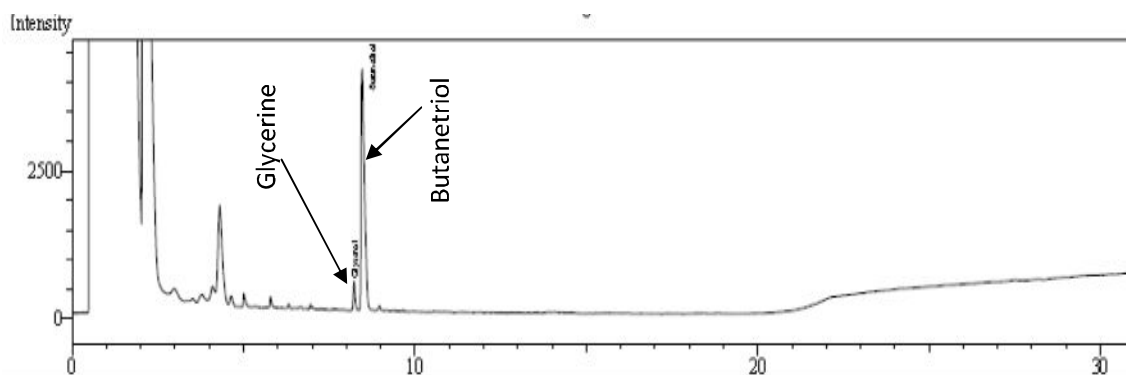


Figure 2 Chromatograms for Glycerine and 1, 2, 3-butanetriol

Data calculated from these chromatograms were used to calibrate the glycerine, mono-, di- and triglycerides curves. Results showed excellent linearity for all the four compounds; the correlation coefficient (R^2) exceeded the 0.99 specifications in ASTM D6584 and EN14015. Tables 2 and 3 show the regression data of the calibration functions for JME and CME, respectively. The correlations show excellent fits of the calibration data which also agrees with what is reported in literature [20,21].

Table 2: Retention time and regression coefficients for JME

	Retention time (min)	a	b	R^2
Glycerine	3.62 – 3.66	0.9884	0.0013	0.9999
Monolein	15.31 – 15.75	1.1794	-0.0264	0.9991
Dioline	21.12 – 21.23	0.9629	0.0078	0.9981
Triolein	24.1 – 24.8	1.0004	-0.00005	1.0000

Table 3: Retention time and regression coefficients for CME

	Retention time (min)	a	b	R^2
Glycerine	2.8 – 3.1	0.9874	0.0017	1.0000
Monolein	17.2 – 17.5	1.0000	-0.00003	0.9998
Dioline	20.6- 20.8	0.9975	-0.00007	0.9999
Triolein	21.6 – 21.7	1.0001	-0.00002	1.0000

Figures 3 and 4 show sample chromatograms for JME and CME, respectively. All the peaks were clearly developed and are comparable to those found in literature [21].

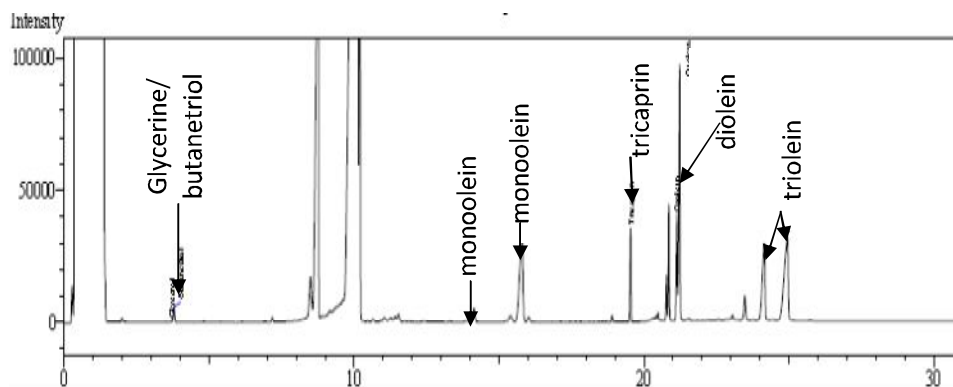


Figure 3 Chromatograms for JME

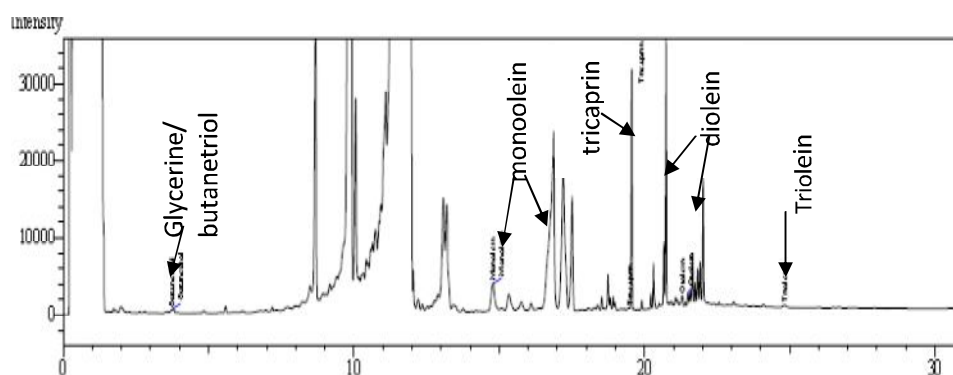


Figure 4 Chromatograms for CME

Jatropha methyl ester results: Amounts of free glycerine, mono-, di- and triglycerides are given as averages in Table 4 for JME. Generally results showed higher amounts of GL (0.10% against 0.03 max allowable), DG (1.10% against 0.16 max allowable), TG (0.98% against 0.14 max allowable) and total glycerides (2.30% against 0.21 max allowable) in EN 14105. ASTM D6584 allowable values are only given for free GL (0.02 max) and total glycerine (0.24 max). However, the amounts of MGs agreed with the standards specified by EN 14105 (0.05% on average against 0.73 max allowable) (Table 4). Repeatability was consistent for GL and MG except for DG, TG and therefore total glycerides. This may indicate incomplete conversion under the studied conditions, especially the catalyst loading of 0.5% instead of the commonly reported value of 1% in literature. It is also possible that during the GC analysis, other materials may co-elute with the glycerol peaks due to the high temperature involved and falsely giving higher results than expected. This phenomenon was also reported by [14]. Peak identifications and calculations were done manual and this could also be a source of the error in the determination of the amounts of glycerides.

Table 4: Concentrations of free glycerine, mono-, di- and triglycerides in JME

Compound	35°C	45°C	55°C	65°C	EN14214‡
GL [%m/m]	0.105	0.110	0.115	0.098	0.01
R*	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	±0.008
MG [%m/m]	0.02	0.07	0.02	0.07	0.73
R	0.06±0.02	0.05±0.02	0.05±0.02	0.11±0.07	±0.063
DG [%m/m]	4.89	1.91	0.50	0.98	0.16
R	1.99±0.74	1.38±0.67	1.01±0.59	0.63±0.53	±0.009
TG [%m/m]	4.01	1.47	0.67	0.82	0.14
R	0.49±0.23	0.35±0.18	0.25±0.12	0.18±0.09	±0.012
T.GL [%m/m]	9.02	3.56	1.31	1.96	0.21
	3.24±1.37	2.26±1.18	1.66±0.97	1.59±1.18	±0.014

R* - Repeatability; ‡[22,23]

Castor methyl ester results: The amounts of contaminants remaining in the products on the average were still high; GL (0.14% against 0.01 max allowable), MG (1.00 against 0.73 max allowable), DG (7.52 against 0.16 max allowable), and TG (2.10% against 0.14 max allowable) compared to the EN14105 standard (Table 5). Since conversions were relatively high (98% wt), this could be a result of insufficient purification of the CME. However, repeatability is within the range of specification for all except DG, TG and therefore total glycerides. CME purification was done by washing the methyl ester with warm water (50°C) and drying on a hot plate (110°C) for 30 minutes.

Table 5: Concentrations of free glycerine, mono-, di- and triglycerides in CME

Compound	35°C	45°C	55°C	65°C	EN14105‡
GL [%m/m]	0.14	0.13	0.14	0.16	0.01
R*	0.00±0.001	0.00±0.001	0.00±0.001	0.008±0.001	±0.008
MG [%m/m]	1.11	1.23	4.20	0.67	0.73
R	-0.03±0.05	-0.03±0.02	-0.06±0.05	0.02±0.00	±0.063
DG [%m/m]	3.86	13.68	7.75	10.96	0.16
R	0.52±0.48	0.78±0.46	1.46±0.45	1.94±0.28	±0.009
TG [%m/m]	6.35	1.97	1.64	2.66	0.14
R	0.41±0.06	0.34±0.22	0.19±0.04	0.22±0.04	±0.012
T.GL [%m/m]	11.46	17.00	13.73	14.44	0.21
R	1.92±0.34	2.01±0.29	2.45±0.37	2.39±0.19	±0.014

R* - Repeatability; ‡ [22,23]

This alone might not have produced sufficient separation of CME from glycerine. Stavarache *et al.*, [24] reported that during washing, soap in the ester tends to accumulate at the interfacial region between the water and the ester; the soap molecules orientate themselves perpendicularly between the two immiscible phases forming an emulsion and therefore making separation difficult. Difficulties in separation of CME have been sufficiently reported in literature [25,26]. A better method of separation for CME would be molecular distillation where a liquid component evaporates without boiling due to high vacuum. Errors could also have come from the laboratory protocol since identification and calculations were done manually. Lack of well trained technicians is still something to be handled within our laboratories.

Conclusion

The GC results gave qualitative and quantitative information. Although some results were not in good agreement with EN 14214 and ASTM D6751 standard specifications, repeatability was in good agreement for both types of oil. This method has been used for quality control of fatty acid methyl esters in production and in compliance with the specified standards.

In the calibration standards, glycerine and butanetriol are masked by the presence of mono, di- and triolein and therefore they need to be run separately but under the same conditions as for mono- di- and triolein. Quantitative determination of monoglyceride, diglyceride and triglycerides was possible by the use of internal standards. The results indicated that jatropha transesterification did not reach completion. This could be due to low catalyst loading and evaporation of methanol. Castor methyl ester results indicated complete conversion but poor purification method.

It is recommended that jatropha transesterification be done using catalyst concentration of 1% wt of oil. Some advanced method of purification like molecular distillation should be use for castor methyl ester.

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