

Characterization of Crude Glycerol from Biodiesel Plants

Shengjun Hu, Xiaolan Luo, Caixia Wan, and Yebo Li*

Department of Food, Agricultural, and Biological Engineering, The Ohio State University/Ohio Agricultural Research and Development Center, 1680 Madison Avenue, Wooster, Ohio 44691-4096, United States

ABSTRACT: Characterization of crude glycerol is very important to its value-added conversion. In this study, the physical and chemical properties of five biodiesel-derived crude glycerol samples were determined. Three methods, including iodometric–periodic acid method, high performance liquid chromatography (HPLC), and gas chromatography (GC), were shown to be suitable for the determination of glycerol content in crude glycerol. The compositional analysis of crude glycerol was successfully achieved by crude glycerol fractionation and characterization of the obtained fractions (aqueous and organic) using titrimetric, HPLC, and GC analyses. The aqueous fraction consisted mainly of glycerol, methanol, and water, while the organic fraction contained fatty acid methyl esters (FAMES), free fatty acids (FFAs), and glycerides. Despite the wide variations in the proportion of their components, all raw crude glycerol samples were shown to contain glycerol, soap, methanol, FAMES, water, glycerides, FFAs, and ash.

KEYWORDS: *characterization, crude glycerol, biodiesel, glycerol, soap, methanol, FAMES, FFAs*

■ INTRODUCTION

With global concerns increasing over the depletion of fossil fuels, the demand for alternative energy sources has been more urgent than ever. Biodiesel is a promising renewable fuel that is mainly produced from the transesterification of vegetable oils or animal fats with methanol catalyzed by alkalis such as NaOH and KOH.^{1,2} After the transesterification process, two layers are formed: the top layer is the desired product, i.e., biodiesel, and the bottom layer is the raw/unrefined crude glycerol.³ The produced biodiesel consists mainly of a mixture of methyl esters of long-chain fatty acids, which can be used directly in compression ignition engines.^{2,4}

Crude glycerol is a major byproduct from the biodiesel production process. It was estimated that approximately 1 kg of crude glycerol is generated for every 10 kg of biodiesel produced.^{5,6} With the rapid growth of the world's biodiesel production in recent years, a large surplus of glycerol has been created,^{5,7} leading to the closure of several traditional glycerol production plants.⁸ At present, crude glycerol is of little economic value, i.e., approximately \$0.1/kg,⁵ due to the presence of various impurities such as methanol, soap, fatty acid methyl esters (FAMES, i.e., biodiesel), and alkaline catalyst residues.^{9,10} It has become a serious issue and financial and environmental liability for the biodiesel industry.⁵ Considerable research has been conducted to develop both chemical and biological processes for the value-added conversion of crude glycerol. Several reviews have been published on this topic.^{5,9,11–13} Some examples of glycerol-derived products are acrolein,^{14–16} 1, 3-propanediol,^{17,18} docosahexaenoic acid (DHA),^{19,20} hydrogen,^{21,22} bio-oil,^{23–25} polyols and polyurethane foams,¹⁰ and polyhydroxylalkanoates.^{26,27} However, most of these processes are still in the research and development stage. Currently, one of the major challenges for the utilization of crude glycerol is the inconsistency of its composition since it varies with the feedstocks, production processes, and post-treatments involved in biodiesel production. Upgrading or refining crude glycerol to technical grade

glycerol (>98% glycerol content) makes its composition more consistent, but currently this is not economically viable, especially for small- and/or medium-sized biodiesel plants.²⁸

Compared to the extensive research reports on the utilization of crude glycerol, few reports have been found on the characterization of crude glycerol. Thompson and He⁶ characterized seven crude glycerol samples prepared from six different types of seed oil feedstocks and one waste cooking oil using the same production practices. The determined properties of crude glycerol included viscosity; heat of combustion; glycerol, methanol, and elemental contents; and food nutrition values. The results showed that little variation existed in the physical and chemical properties of different crude glycerol samples. Hansen et al.²⁹ characterized 11 crude glycerol samples collected from different biodiesel plants in Australia. Several components of crude glycerol including glycerol, moisture, ash, methanol, and MONG (matter organic non-glycerol) were characterized. In contrast to the findings of Thompson and He, the results of Hansen et al. showed that the properties of different crude glycerol samples varied significantly from each other, which were mostly likely caused by the various biodiesel production processes used in different biodiesel plants.

In addition to the crude glycerol components determined by the above two studies, raw/unrefined crude glycerol from biodiesel plants also contains other components such as soap, free fatty acids (FFAs), FAMES, and glycerides. The impurities present in crude glycerol significantly affect its properties and thus its conversion to value-added products. For example, soap and methanol have been found to negatively influence the algal production of DHA from crude glycerol,²⁰ and the high salinity (Na or K) of crude glycerol can largely inhibit the microbial

Received: February 28, 2012

Revised: May 15, 2012

Accepted: May 21, 2012

Published: May 21, 2012

activity in the anaerobic digestion of crude glycerol.⁹ In addition, for pharmaceutical and food applications, heavy metals in crude glycerol might be harmful and present safety concerns.²⁰ In contrast, some studies also indicated that certain crude glycerol impurities can help improve the properties of crude glycerol-based products such as bio-oil³⁰ and polyurethane foams.¹⁰ Therefore, considering the significant impacts of crude glycerol impurities on its value-added processing, it is vital to understand the chemical composition of crude glycerol before considering value-added conversions. To our knowledge, there have been no studies dedicated to a comprehensive compositional analysis, i.e., identification of all chemical components, of biodiesel-derived crude glycerol. In this study, due to the complex nature of its composition, crude glycerol was treated and fractionated into aqueous and organic fractions in order to better characterize its individual components. Each fraction was then characterized via a series of analytical techniques. The main purposes of this study were to (1) obtain a complete chemical compositional profile of the biodiesel-derived crude glycerol; and (2) evaluate different analytical methods for the characterization of the biodiesel-derived crude glycerol.

MATERIALS AND METHODS

Materials. Five crude glycerol samples (soybean oil based CG-Soy1, CG-Soy2, and CG-Soy3; waste vegetable oil based CG-WV; and soybean oil–waste vegetable oil mixture based CG-SW) were provided by PolyGreen Technologies LLC (Mansfield, OH). Chemicals purchased from Fisher Scientific (Pittsburgh, PA) included bromophenol blue (sodium salt), potassium iodide, potassium hydroxide, phenolphthalein, periodic acid, pure glycerol, trace metal grade HNO₃, concentrated HCl (35–38% wt.), standard HCl (0.1 N), NaOH (1 N), Na₂S₂O₃ (1 N), 0.1% w/v methyl orange, and 1% w/v starch indicator solution. Chemicals purchased from Sigma Aldrich (St. Louis, MO) included anhydrous hexane, anhydrous Na₂SO₄, methyl heptadecanoate, standard FAME mix (GLC-10), HPLC (High performance liquid chromatography) grade H₂SO₄ (50% wt.), and 10% wt BF₃–methanol. Chloroform, glacial acetic acid, ethanol, methanol, and petroleum ether were purchased from Pharmco-AAPER (Shelbyville, KY). Standard stock solutions of monoolein, diolein, triolein, 1,2,4-butanetriol, tricaprin, and derivitization reagent *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) were purchased from Restek (Bellefonte, PA). All chemicals used were of reagent grade or higher purity.

Physical Properties. The density of crude glycerol was determined by measuring the volume and weight of crude glycerol at room temperature (23 ± 0.5 °C). For pH determination, crude glycerol (1.00 ± 0.1 g) was dissolved in 50 mL of deionized (DI) water. The pH of the solution was measured by a digital pH meter (Oakton pH 11 series, South Burlington, VT) at room temperature (23 ± 0.5 °C). The viscosity of crude glycerol was measured at 25 ± 0.5 °C according to ASTM D 4878-08³¹ using a Brookfield DV II+Pro viscometer equipped with a small sample adapter, a temperature probe, and a temperature control unit (Brookfield Engineering Laboratories, Inc., Middleboro, MA).

Soap Content Determination. The soap content of crude glycerol was determined with reference to AOCs Recommended Practice Cc 17-95³² and ASTM D 4662-08.³³ Briefly, the unadjusted soap content of crude glycerol was determined according to AOCs Recommended Practice Cc 17-95. The alkalinity of crude glycerol was determined according to ASTM D 4662-08³³ and used to adjust the soap content. The adjusted soap content of crude glycerol was calculated as follows:

$$\text{Soap as sodium oleate, \%} = (V_s - V_a) \times N \times 30.44/W$$

where V_s = mL of titrant consumed, soap titration; V_a = mL of titrant consumed, alkalinity titration; N = normality, HCl solution; W = mass (g) of crude glycerol weighed.

Water and Ash Content Determination. The water content of crude glycerol was determined by volumetric Karl Fischer titration using a T70 automatic titration system (Mettler Toledo, Columbus, OH) with reference to AOCs Official Method Ea 8-58.³⁴ The ash content of crude glycerol was determined by burning approximately 1 g of sample in a furnace at 750 °C for 3 h.²⁸

Elemental Analysis. The C and N contents of crude glycerol were determined by VarioMax CNS analyzer (Mt. Laurel, NJ). Elements including Na, Mg, Al, P, K, Ca, Mn, Fe, Co, Cu, and Zn were determined by ICP-MS (Agilent Technologies ICP-MS 7500 series, Santa Clara, CA). Crude glycerol (0.20–0.50 g) was digested using 10 mL of trace metal grade HNO₃ in a microwave digester (MARSXpress, CEM Corporation, Matthews, NC). The digester temperature was ramped to 200 °C in 15 min, maintained at 200 °C for 15 min, and cooled down to 25 °C. The digested crude glycerol solution was transferred into a 500-mL volumetric flask and filled to mark using Mini-Q water (Synergy UV, Millipore Corp., Billerica, MA). The solution was mixed and analyzed using ICP-MS.

Glycerol Content Determination by Iodometric–Periodic Acid Method. The determination of the free glycerol content of crude glycerol by iodometric–periodic acid method was conducted with reference to AOCs Official Method Ca 14-56.³⁵

Fractionation of Crude Glycerol. Crude glycerol was fractionated into aqueous and organic fractions according to the procedures

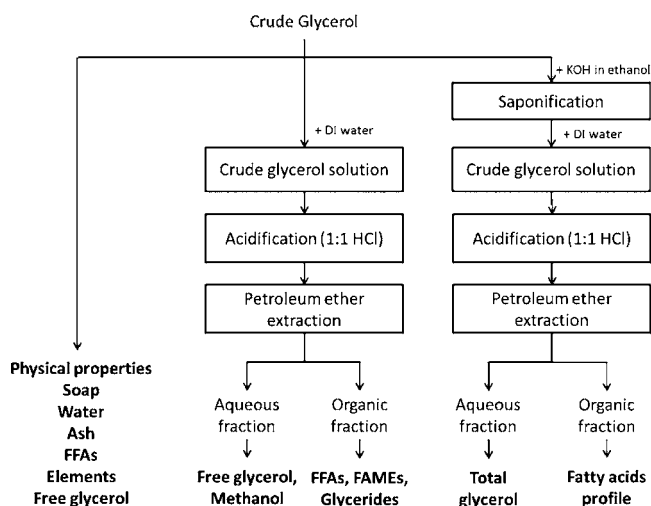


Figure 1. Scheme of crude glycerol fractionation and characterization.

shown in Figure 1. For fractionation without saponification, the crude glycerol sample was weighed and well dissolved in 50 mL of DI water in a 120-mL pressure tube (pressure rating: 1 MPa, ACE Glass Inc. Vineland, NJ). The solution was then acidified and fractionated with reference to AOCs Official Method G 3-53.³⁶ Briefly, complete conversion of soap in crude glycerol to FFAs was achieved by acidifying a crude glycerol solution using a sufficient amount (5 mL) of 1:1 (v/v) HCl solution, and then the recovery of the organic fraction was achieved by petroleum ether extraction. To ensure the complete recovery of the organic fraction, petroleum ether extraction was repeated until the obtained top petroleum ether layer became colorless. The organic fraction was obtained after the removal of petroleum ether by rotary-evaporation (Laborota efficient 4001, Heidolph, Schwabach, Germany) and vacuum-drying until a constant weight was reached (Isotemp 282A, Fish Scientific, Pittsburgh, PA). For fractionation with saponification, the crude glycerol sample was weighed into a 120-mL pressure tube (ACE Glass Inc. Vineland, NJ) and dissolved in 10 mL of 50 g/L KOH-ethanol solution. The tight-capped tube was heated in an oil bath at 90 °C for 1 h with constant

magnetic stirring (200 rpm). Upon completion, the tube was removed from the bath and cooled to room temperature (23 ± 0.5 °C) in a fume hood, followed by the addition of 50 mL of DI water. The solution was then acidified and fractionated using the procedures as described in fractionation without saponification. All aqueous and organic fractions obtained from the fractionation of crude glycerol were kept for later analyses.

HPLC Analysis of Glycerol and Methanol. The free glycerol and methanol contents of crude glycerol were determined by HPLC analysis using aqueous fractions obtained from crude glycerol fractionation without saponification. The total glycerol content of crude glycerol was determined by HPLC analysis using aqueous fractions obtained from crude glycerol fractionation with saponification. Aqueous fraction samples were filtered and analyzed using a LC-20 AB HPLC system (Shimadzu, Columbia, MD) equipped with a RID-10A refractive index detector and a RFQ-Fast Fruit H+ (8%) column (Phenomenex, Torrance, CA). The mobile phase used was 0.005 N H₂SO₄ at a flow rate of 0.6 mL/min. The column and RID temperatures were maintained at 60 and 55 °C, respectively. The injection volume was 10 μ L. An external calibration curve was constructed by analyzing standard glycerol and methanol solutions at different concentration levels.

GC Analysis of Glycerides, Glycerol, FAMES, and FFAs. The free glycerol in crude glycerol, the FAMES and glycerides in organic fractions obtained without saponification, and the fatty acid profiles of the free fatty acids (FFAs) in organic fractions obtained with saponification were determined by gas chromatography (GC) using a Shimadzu GC-2010 plus GC system (Shimadzu, Columbia, MD) equipped with a flame ionization detector (FID). The GC analysis of glycerides was conducted according to ASTM D6584-10a.³⁷

The GC analysis of the free glycerol content of crude glycerol was conducted as follows: weighed crude glycerol (40.0–100.0 mg) was acidified by 100 μ L 1:1 HCl (v/v) and then dissolved in 10 mL of pyridine in a 15-mL glass test tube (Pyrex, Corning, NJ). Then, an aliquot of the obtained solution and 100 μ L of 1,2,4-butanetriol standard solution (0.89 mg/mL, internal standard) were mixed and derivatized by MSTFA (100 μ L) at 38 °C for 15 min. The sample was then filtered and injected at an injection volume of 1 μ L into a MXT-Biodiesel TG column (14 m, 0.53 mm, 0.16 μ m, Restek, Bellefonte, PA). Helium was used as the carrier gas at a flow rate of 3 mL/min. The injector and column temperatures were ramped from 50 to 110 at 5 °C/min with the detector temperature held constant at 380 °C. A calibration curve was constructed by analyzing pure glycerol at different concentration levels.

For FAME analysis, the organic fraction sample (without saponification) was weighed (20–50 mg) and dissolved in 5 mL of anhydrous hexane in a 15-mL glass test tube (Pyrex, Corning, NJ). An aliquot of the obtained solution and 100 μ L of methyl heptadecanoate standard solution (internal standard) were mixed and filtered through a PTFE filter (porosity, 0.22 μ m; diameter, 13 mm, Fisher Scientific, Pittsburgh, PA) into a 1.5-mL GC vial. Each sample (1 μ L) was injected into a Stabliwax-DA column (30 m, 0.32 mm id, 0.5 μ m df, Restek, Bellefonte, PA) at an injection temperature of 200 °C. The column temperature was ramped from 100 to 250 at 5 °C/min with detector temperature held constant at 250 °C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The calibration curve was constructed by analyzing standard FAME solutions at different concentration levels.

The fatty acid profiles of the FFAs in organic fractions (with saponification) were analyzed as follows: sample (60–150 mg) was weighed into a Teflon-capped test tube, followed by the addition of 2 mL of 10% w/w BF₃–methanol. The tube was heated in a water bath maintained at 60 °C for 15 min, after which 1 mL of hexane and 1 mL of water were added. The top hexane phase containing methyl esters derivatives of FFAs was analyzed by GC according to the FAME analysis procedure described above.

Titrimetric Determination of FFA Content. The FFA contents of crude glycerol and of organic fractions (without and with saponification) were determined with reference to AOCS Official Method Ca 5a-40.³⁸

Statistical Analysis. Statistical analysis of the obtained data was conducted by IBM SPSS Statistics 19 (Armonk, NY). The factor significance was tested by an ANOVA procedure at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Physical Properties of Crude Glycerol. The density of five crude glycerol samples varied between 1.01 and 1.20 g/cm³, lower than that of pure glycerol (1.31 g/cm³) (Table 1), due to

Table 1. Physical Properties of Crude Glycerol^a

| samples | density (g/cm ³) | pH | viscosity (mPa·s) |
|---------------|------------------------------|----------------|-------------------|
| CG-Soy1 | 1.20 \pm 0.01 | 6.9 \pm 0.0 | 15 \pm 0.1 |
| CG-Soy2 | 1.02 \pm 0.02 | 9.7 \pm 0.0 | 162 \pm 11 |
| CG-Soy3 | 1.01 \pm 0.00 | 9.5 \pm 0.0 | 110 \pm 7 |
| CG-WV | 1.01 \pm 0.00 | 9.4 \pm 0.0 | 1213 \pm 129 |
| CG-SW | 1.11 \pm 0.01 | 10.0 \pm 0.0 | 838 \pm 28 |
| pure glycerol | 1.31 \pm 0.00 | 6.4 \pm 0.0 | 930 \pm 12 |

^aData expressed as mean of three replicates \pm standard deviation.

the presence of some lighter impurities such as fatty acids methyl esters (FAMES), fatty acids, methanol, and water in crude glycerol. All samples, except CG-Soy1, had pH values close to 10 because of the existence of residual alkalis such as NaOH or KOH left from the biodiesel production process. CG-Soy1 had a lower pH value (6.9), which was close to that of pure glycerol (6.4), due to the removal of residual alkalis by some post-treatments in the biodiesel plant. In the study of Hansen et al., the pH values of the 11 crude glycerol samples from different biodiesel plants in Australia varied from 2.0 to 10.8.²⁹ The viscosity of five crude glycerol samples ranged between 15 and 1213 mPa·s, due to their different compositions. Waste vegetable oil based crude glycerol (CG-WV) had the highest viscosity of 1213 mPa·s.

Comparative Glycerol Determination in Crude Glycerol. The quantification of glycerol can be achieved by a variety of analytical techniques such as the titrimetric method, HPLC, and GC.^{20,35,37} In this study, the feasibility of applying these three methods to the determination of glycerol in crude glycerol was explored. The free glycerol contents of CG-WV determined by iodometric–periodic acid method, HPLC, and GC were 27.6, 27.9, and 27.4%, respectively (Table 2), which were comparable to each other. The relative standard deviations (% RSD) of all three methods were below 2%. All

Table 2. Comparative Determination of the Free Glycerol Content of CG-WV

| replicates | glycerol content (wt %) | | |
|----------------------|-------------------------|------|------|
| | iodometry ^d | HPLC | GC |
| 1 | 27.5 | 27.7 | 27.5 |
| 2 | 28.1 | 27.9 | 27.7 |
| 3 | 26.9 | 27.8 | 27.2 |
| 4 | 28.3 | 27.9 | 27.5 |
| 5 | 27.0 | 28.3 | 27.6 |
| 6 | 27.8 | 27.8 | 26.9 |
| 7 | 27.9 | 28.0 | 27.3 |
| mean ^a | 27.6 | 27.9 | 27.4 |
| Std. ^b | 0.5 | 0.2 | 0.3 |
| RSD ^c (%) | 1.9 | 0.7 | 1.0 |

^aMean of seven replicates. ^bStandard deviation. ^cRelative standard deviation. ^dIodometric–periodic acid method.

following glycerol contents reported in this study were determined using HPLC since it allows the simultaneous determination of glycerol and methanol contents in crude glycerol.

Glycerol and Methanol in Crude Glycerol. Figure 2 shows a representative HPLC chromatogram for the determi-

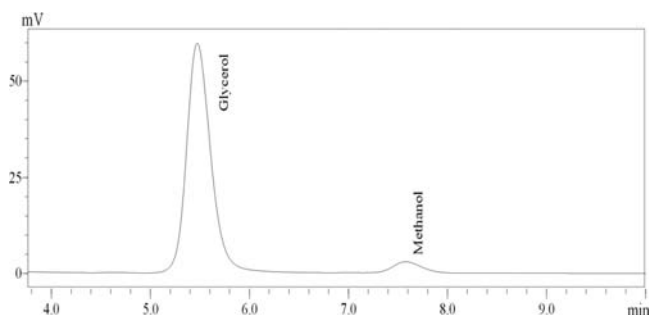


Figure 2. Representative HPLC chromatogram for the determination of glycerol and methanol in crude glycerol.

nation of glycerol and methanol in crude glycerol. It can be seen that the separation and elution of glycerol and methanol were achieved within 10 min. The glycerol determined using aqueous fractions obtained without and with saponification was referred to as free and total glycerol in crude glycerol, respectively. During the saponification process, glycerides in crude glycerol, if any, were converted to glycerol and soap (Figure 3b). Therefore, the glycerol in aqueous fractions with

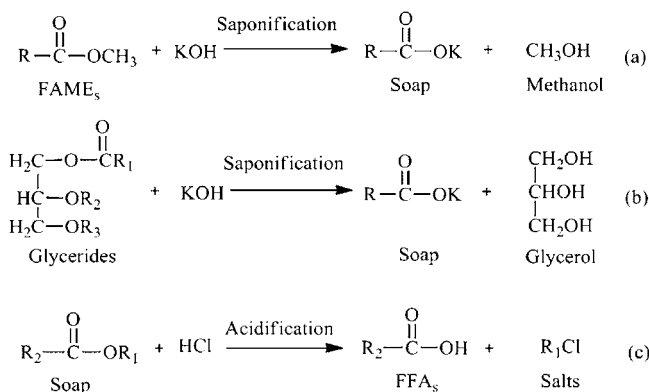


Figure 3. Chemical reactions occurring in crude glycerol fractionation processes: (a) R, aliphatic tails; (b) R₁, aliphatic tails; R₂ and R₃, -H or -COR (R, aliphatic tails); (c) R₁, Na or K; R₂, aliphatic tails.

saponification included not only the free glycerol that originally existed in crude glycerol but also the combined glycerol

released from glycerides by saponification. As shown in Table 3, the free glycerol contents of five crude glycerol samples varied from 22.9 to 63.0%, which were significantly different from each other ($p < 0.05$). The glycerol contents of the 11 crude glycerol samples in the study of Hansen et al. varied from 38.4 to 96.5%.²⁹ In contrast, the glycerol contents of the crude glycerol prepared in the lab using seven different seed oils in Thompson and He's study⁶ varied less significantly from 62.5 to 76.6%. For all crude glycerol samples except CG-WV, the total glycerol contents were close to their respective free glycerol contents (Table 3). A statistical analysis showed that there were no significant differences ($p > 0.05$) between these two types of glycerol contents, indicating the low contents of combined glycerol, i.e., glycerides, in crude glycerol. CG-WV showed a total glycerol content (28.9%) slightly higher than its free glycerol content (27.9%), indicating its higher content of glycerides.

The methanol contents of five crude glycerol samples varied significantly ($p < 0.05$), ranging from 6.2 to 12.6% (Table 3). During the biodiesel production process, an excess of methanol is usually used to increase biodiesel yield.¹ After the production process, excess methanol is generally recovered by a distillation process.³ The residual methanol in crude glycerol varies with the methanol inputs and post recovery efficiencies in different biodiesel plants.

FFAs, FAMES, and Glycerides in Organic Fractions.

The compositions of the organic fractions obtained without saponification were determined in terms of their FFA, FAME, and glyceride contents. For crude glycerol fractionation without saponification, the organic fraction contents of five crude glycerol samples varied from BDL (below detection limit) to 55.8% (Table 3). CG-Soy1 showed no detectable levels of organic fraction because it was post-treated and refined to remove most of its organic impurities. Figures 4 and 5 show

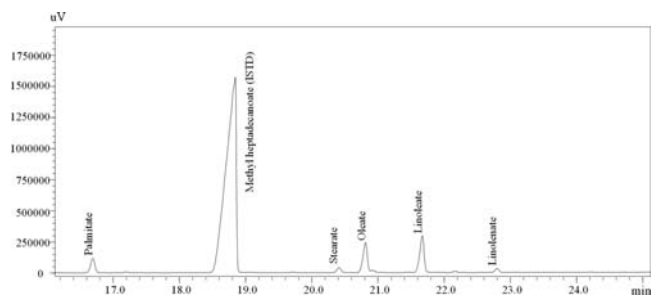


Figure 4. Representative GC chromatogram for the determination of FAMES in organic fractions obtained without saponification.

representative GC chromatograms for the determination of FAMES and glycerides in the obtained organic fractions,

Table 3. Glycerol, Methanol, and Organic Fraction Contents of Crude Glycerol^a

| samples | without saponification | | with saponification | | |
|---------|------------------------|--------------------------------------|-----------------------|-------------------------|-----------------|
| | free glycerol (wt %) | organic fraction ^b (wt %) | total glycerol (wt %) | organic fraction (wt %) | methanol (wt %) |
| CG-Soy1 | 63.0 ± 0.3 | BDL ^c | 63.3 ± 0.7 | BDL | 6.2 ± 0.0 |
| CG-Soy2 | 22.9 ± 0.2 | 45.0 ± 0.3 | 22.9 ± 0.1 | 43.1 ± 0.5 | 10.9 ± 0.2 |
| CG-Soy3 | 33.3 ± 0.1 | 43.4 ± 0.7 | 33.3 ± 0.3 | 40.5 ± 0.5 | 12.6 ± 0.1 |
| CG-WV | 27.9 ± 0.2 | 55.8 ± 0.7 | 28.9 ± 0.6 | 52.3 ± 0.3 | 8.6 ± 0.0 |
| CG-SW | 57.1 ± 0.0 | 26.1 ± 0.5 | 57.3 ± 0.6 | 26.0 ± 0.1 | 11.3 ± 0.0 |

^aData are expressed as the mean of three replicates ± standard deviation. ^bMainly consist of FAMES, FFAs, and glycerides. ^cBelow the detection limit.

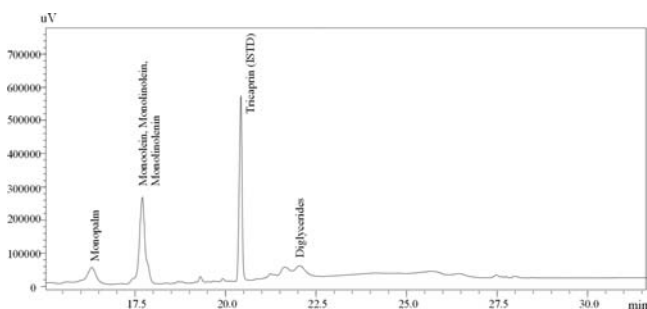


Figure 5. Representative GC chromatogram for the determination of glycerides in organic fractions obtained without saponification.

respectively. It can be seen that the separation of FAMES and glycerides was effective. The FFA contents of the organic fractions of four crude glycerol samples ranged between 35.7 and 96.4% (Table 4). The FFAs determined in organic fractions included not only FFAs originally existed in crude glycerol but also those converted from soap by acidification (Figure 3c).^{20,30} The existence of soap in crude glycerol can be attributed to the occurrence of saponification, an unfavorable side reaction, during the biodiesel production process.¹ The total FAME contents (sum of all individual methyl esters) of the organic fractions of four crude glycerol samples ranged between 5.2 and 51.6%. The presence of FAMES in crude glycerol was most likely caused by the incomplete separation between ester and glycerol layers during the biodiesel production process.¹ Compared to FFAs and FAMES, glycerides were found to have relatively low contents (<5%) in organic fractions, except for CG-WV (12.6%). The low glyceride contents of CG-Soy2, CG-Soy3, and CG-SW corresponded well with the above results that the free glycerol contents were similar to the total glycerol contents (Table 3). The higher glyceride content of CG-WV led to a total glycerol content higher than its free glycerol content (Table 3), due to the release of combined glycerol as discussed before. A statistical analysis showed that the compositions of the organic fractions (i.e., the contents of FFAs, FAMES, and glycerides) of four crude glycerol samples were significantly different from each other ($p < 0.05$).

For crude glycerol fractionation with saponification, the obtained organic fractions of five crude glycerol samples varied between BDL to 52.3% (Table 3). FFA analysis showed that all obtained organic fractions had FFA contents around 100% (data not shown), indicating that they consisted mainly of FFAs. Therefore, the saponification treatment significantly

changed the compositions of the obtained organic fractions. This is probably because the saponification process converted FAMES and/or glycerides in crude glycerol to soap and other compounds, and the soap was further converted to FFAs by acidification (Figure 3). The saponification process also resulted in CG-Soy2, CG-Soy3, and CG-WV having lower organic fraction contents as compared to those without the saponification process (Table 3), due to the weight losses of organic fractions associated with the conversion of FAMES and/or glycerides to FFAs. CG-SW showed no significant difference between the contents of these two types of organic fractions (Table 3) due to its low content of FAMES and glycerides (Table 4). The fatty acid profile analyses of the FFAs in organic fractions showed that all samples had high contents of oleic (23.3 to 35.2%), linoleic (25.6 to 46.8%), and palmitic acids (10.0 to 12.9%) (Table 5), which were similar to the fatty acid profiles of soybean and some other vegetable oils.³⁹

Table 5. Composition of Organic Fractions Obtained with Saponification^a

| samples | CG-Soy2 | CG-Soy3 | CG-WV | CG-SW |
|----------------------------|------------|------------|------------|------------|
| palmitic acid (wt %) | 12.9 ± 1.0 | 10.4 ± 1.4 | 14.1 ± 0.5 | 10.0 ± 1.7 |
| stearic acid (wt %) | 4.6 ± 0.6 | 3.5 ± 0.8 | 6.3 ± 0.1 | 1.2 ± 0.3 |
| oleic acid (wt %) | 35.2 ± 2.7 | 25.9 ± 3.9 | 29.3 ± 0.3 | 23.3 ± 4.1 |
| linoleic acid (wt %) | 37.6 ± 2.9 | 36.0 ± 3.4 | 25.6 ± 1.5 | 46.8 ± 8.6 |
| linolenic acid (wt %) | 4.0 ± 1.1 | 4.5 ± 1.1 | 2.1 ± 1.0 | 1.2 ± 0.2 |
| others ^b (wt %) | 5.7 ± 3.3 | 19.7 ± 4.1 | 22.7 ± 1.0 | 17.5 ± 7.5 |

^aData are expressed as the mean of three replicates ± standard deviation. ^bBalance after the quantification of 5 common FAMES.

Elemental Analysis. Table 6 shows that the C and N contents of five crude glycerol samples ranged from 24.3 to 54.2% and from 0.3 to 1.2%, respectively. The C and N contents of seven crude glycerol samples evaluated in Thompson and He's study⁶ ranged from 24.0 to 37.7% and 0.04 to 0.12%, respectively. The higher C content of crude glycerol obtained in this study can be explained by the higher contents of certain impurities in crude glycerol such as soaps, FAMES, and glycerides, which have higher C contents than glycerol. The high content of K in CG-SW and Na in CG-Soy1, CG-Soy2, CG-Soy3, and CG-WV can be attributed to the use

Table 4. Composition of Organic Fractions Obtained without Saponification^a

| samples ^b | | CG-Soy2 | CG-Soy3 | CG-WV | CG-SW |
|--------------------------------|---------------------|------------|------------|------------|------------------|
| FFAs (wt %) | | 50.0 ± 0.2 | 51.6 ± 0.1 | 35.7 ± 0.6 | 96.4 ± 0.4 |
| glycerides ^c (wt %) | mono- | 1.6 ± 0.1 | 2.2 ± 0.4 | 5.2 ± 0.3 | 0.7 ± 0.0 |
| | di- | 1.1 ± 0.2 | 1.6 ± 0.1 | 7.4 ± 3.3 | 0.9 ± 0.0 |
| | | | | | |
| FAMES (wt %) | palmitate | 4.9 ± 0.2 | 4.5 ± 0.1 | 6.9 ± 0.2 | 0.3 ± 0.1 |
| | stearate | 1.8 ± 0.1 | 1.5 ± 0.1 | 2.8 ± 0.2 | BDL ^e |
| | oleate | 14.0 ± 0.5 | 11.0 ± 0.2 | 14.7 ± 0.6 | 0.8 ± 0.2 |
| | linoleate | 14.8 ± 0.6 | 14.2 ± 0.4 | 12.7 ± 0.7 | 2.7 ± 0.0 |
| | linolenate | 1.9 ± 0.2 | 2.0 ± 0.1 | 1.3 ± 0.1 | 1.4 ± 0.7 |
| | others ^d | 9.9 ± 1.3 | 11.3 ± 0.7 | 13.2 ± 2.5 | BDL |

^aData are expressed as the mean of three replicates ± standard deviation, and all percentages were expressed on the weight basis of organic fractions. ^bCG-Soy1 is not listed because of no detectable organic fractions. ^cTriglycerides are below the detection limit for all CG samples. ^dBalance after quantification of FFAs, glycerides, and 5 common FAMES. ^eBelow detection limit.

Table 6. Elemental Analysis of Crude Glycerol^a

| samples | CG-Soy1 | CG-Soy2 | CG-Soy3 | CG-WV | CG-SW |
|----------|------------------|--------------|----------------|--------------|--------------|
| C (wt %) | 24.3 ± 0.2 | 44.3 ± 0.4 | 46.8 ± 0.4 | 54.2 ± 0.1 | 42.5 ± 0.3 |
| N (wt %) | 0.3 ± 0.1 | 0.6 ± 0.1 | 0.7 ± 0.1 | 0.9 ± 0.3 | 1.2 ± 0.1 |
| Na (ppm) | 11769 ± 1561 | 19361 ± 2647 | 19108 ± 2480 | 16263 ± 2363 | 90.6 ± 36.0 |
| K (ppm) | 118.8 ± 26.8 | 140.5 ± 23.7 | 1057.0 ± 137.3 | 486.2 ± 64.5 | 39390 ± 3276 |
| P (ppm) | 38.7 ± 4.8 | 101.3 ± 15.5 | 24.7 ± 8.8 | 233.8 ± 33.6 | BDL |
| Ca (ppm) | BDL ^b | 8.4 ± 3.7 | 4.0 ± 4.8 | 20.5 ± 9.5 | 17.4 ± 2.4 |
| Mg (ppm) | BDL | 3.7 ± 0.6 | BDL | 14.2 ± 3.0 | BDL |
| Fe (ppm) | 31.6 ± 19.0 | 34.2 ± 12.0 | 52.8 ± 24.9 | 92.1 ± 33.0 | 37.4 ± 15.6 |

^aData are expressed as the mean of three replicates ± standard deviation. Al, Mn, Co, Cu, and Zn were determined to be below the detection limit and are not listed ^bBelow the detection limit.

Table 7. Composition of Crude Glycerol^a

| samples | CG-Soy1 | CG-Soy2 | CG-Soy3 | CG-WV | CG-SW |
|--------------------------------|------------------|------------|------------|------------|------------|
| free glycerol (wt %) | 63.0 ± 0.3 | 22.9 ± 0.2 | 33.3 ± 0.1 | 27.8 ± 0.2 | 57.1 ± 0.0 |
| methanol (wt %) | 6.2 ± 0.0 | 10.9 ± 0.2 | 12.6 ± 0.1 | 8.6 ± 0.0 | 11.3 ± 0.0 |
| water (wt %) | 28.7 ± 0.3 | 18.2 ± 0.1 | 6.5 ± 0.1 | 4.1 ± 0.1 | 1.0 ± 0.1 |
| soap (wt %) | BDL ^e | 26.2 ± 0.2 | 26.1 ± 0.1 | 20.5 ± 0.1 | 31.4 ± 0.1 |
| FAMES ^b (wt %) | BDL | 21.3 ± 0.2 | 19.3 ± 0.3 | 28.8 ± 1.1 | 0.5 ± 0.1 |
| glycerides ^b (wt %) | BDL | 1.2 ± 0.2 | 1.6 ± 0.3 | 7.0 ± 0.5 | 0.4 ± 0.1 |
| FFAs ^c (wt %) | BDL | 1.0 ± 0.1 | 1.4 ± 0.1 | 3.0 ± 0.1 | BDL |
| ash (wt %) | 2.7 ± 0.1 | 3.0 ± 0.0 | 2.8 ± 0.1 | 2.7 ± 0.0 | 5.7 ± 0.2 |
| total ^d (wt %) | 99.4 | 102.9 | 101.8 | 101.1 | 103.6 |

^aData are expressed as the mean of three replicates ± standard deviation. ^bGlycerides and FAMES contents determined from their known contents in organic fraction (Table 4) and the contents of organic fraction in crude glycerol (Table 3). ^cFFAs originally existed in crude glycerol, not including those converted from soap acidification. ^dSum of all rows minus Na (CG-Soy1, Soy2, Soy3, and WV) or K (CG-SW) in Table 6 since they were double-counted in soap and ash content. ^eBelow the detection limit.

of K- and Na-based alkalis, respectively, during the biodiesel production process.¹

Soap, Water, and Ash in Crude Glycerol. As shown in Table 7, the soap contents of all crude glycerol samples, except CG-Soy1, were relatively high (20.5 to 31.4%), while all crude glycerol samples showed low FFA contents (≤3.0%). This indicates that the FFAs in organic fractions (without saponification, Table 4) were mainly derived from the acidification of the soap that existed in crude glycerol. The water and ash contents of five crude glycerol samples ranged from 1.0 to 28.7% and from 2.7 to 5.7%, respectively. In addition, it can be noted that for each crude glycerol sample, its combined soap, FAMES, and glyceride content (Table 7) was higher than its respective organic fraction content (Table 3), which consisted of FFAs, FAMES, and glycerides. This is due to the fact that soap was converted to FFAs during the acidification process (Figure 3c): soap has a higher molecular weight than its corresponding FFA, and thus, the weight of obtained organic fractions was lower than that of the sum of soap, FAMES, and glycerides in crude glycerol.

Composition of Crude Glycerol. As shown in Table 7, the chemical compositions of all crude glycerol samples, except CG-Soy1 (partially refined), were appropriately described by eight components, i.e., glycerol, methanol, water, soap, FAMES, glycerides, FFAs, and ash. For all crude glycerol samples, the glycerol, methanol, FAMES, soap, and water in total accounted for over 85% of the mass of crude glycerol, while the total of glycerides, FFAs, and ash was generally less than 15% (Table 7). The compositions of five crude glycerol samples varied significantly from each other; for example, glycerol contents ranged from 22.9 to 63.0%. Therefore, it is necessary to

characterize the composition of crude glycerol before considering it for value-added conversions.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 1 330 263 3855. Fax: + 1 330 263 3670. E-mail: li.851@osu.edu.

Funding

This project is supported by funding from the Ohio Soybean Council.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Mary Wicks (Department of Food, Agricultural and Biological Engineering, OSU) for reading through the manuscript and providing useful suggestions.

REFERENCES

- (1) Ma, F. R.; Hanna, M. A. Biodiesel Production: A Review. *Bioresour. Technol.* **1999**, *70*, 1–15.
- (2) Chien, Y.; Lu, M.; Chai, M.; Boreo, F. J. Characterization of Biodiesel and Biodiesel Particulate Matter by TG, TG-MS, and FTIR. *Energy Fuels* **2009**, *23*, 202–206.
- (3) Hajek, M.; Skopal, F. Treatment of Glycerol Phase Formed by Biodiesel Production. *Bioresour. Technol.* **2010**, *101*, 3242–3245.
- (4) Meher, L. C.; Sagar, D. V.; Naik, S. N. Technical Aspects of Biodiesel Production by Transesterification: A Review. *Renewable Sustainable Energy Rev.* **2006**, *10*, 248–268.
- (5) Johnson, D. T.; Taconi, K. A. The Glycerin Glut: Options for the Value-Added Conversion of Crude Glycerol Resulting from Biodiesel Production. *Environ. Prog.* **2007**, *26*, 338–348.

- (6) Thompson, J. C.; He, B. B. Characterization of Crude Glycerol from Biodiesel Production from Multiple Feedstocks. *Appl. Eng. Agric.* **2006**, *22*, 261–265.
- (7) Pagliaro, M.; Ciriminna, R.; Kimura, H.; Rossi, M.; Della Pina, C. From Glycerol to Value-Added Products. *Angew. Chem., Int. Ed.* **2007**, *46*, 4434–4440.
- (8) McCoy, M. Glycerin Surplus: Plants are Closing, and New Uses for the Chemical are being found. *Chem. Eng. News* **2006**, *84*, 7.
- (9) Santibanez, C.; Teresa Varnero, M.; Bustamante, M. Residual Glycerol from Biodiesel Manufacturing, Waste Or Potential Source of Bioenergy: A Review. *Chil. J. Agric. Res.* **2011**, *71*, 469–475.
- (10) Hu, S.; Wan, C.; Li, Y. Production and Characterization of Biopolyols and Polyurethane Foams from Crude Glycerol Based Liquefaction of Soybean Straw. *Bioresour. Technol.* **2012**, *103*, 227–33.
- (11) Pachauri, N.; He, B. ASABE meeting presentation: Value-Added Utilization of Crude Glycerol from Biodiesel Production: A Survey of Current Research Activities; ASABE paper number: 066223. Presented at 2006 ASABE Annual International Meeting, Portland, Oregon, July 9–12, 2006.
- (12) Behr, A.; Eilting, J.; Irawadi, K.; Leschinski, J.; Lindner, F. Improved Utilisation of Renewable Resources: New Important Derivatives of Glycerol. *Green Chem.* **2008**, *10*, 13–30.
- (13) Zhou, C.; Beltrami, J. N.; Fan, Y.; Lu, G. Q. Chemoselective Catalytic Conversion of Glycerol as a Biorenewable Source to Valuable Commodity Chemicals. *Chem. Soc. Rev.* **2008**, *37*, 527–549.
- (14) Ott, L.; Bicker, M.; Vogel, H. Catalytic Dehydration of Glycerol in Sub- and Supercritical Water: A New Chemical Process for Acrolein Production. *Green Chem.* **2006**, *8*, 214–220.
- (15) Chai, S.; Wang, H.; Liang, Y.; Xu, B. Sustainable Production of Acrolein: Investigation of Solid Acid-Base Catalysts for Gas-Phase Dehydration of Glycerol. *Green Chem.* **2007**, *9*, 1130–1136.
- (16) Corma, A.; Huber, G. W.; Sauvanauda, L.; O'Connor, P. Biomass to Chemicals: Catalytic Conversion of glycerol/water Mixtures into Acrolein, Reaction Network. *J. Catal.* **2008**, *257*, 163–171.
- (17) Asad-ur-Rehman; Saman, W. R. G.; Nomura, N.; Sato, S.; Matsumura, M. Pre-Treatment and Utilization of Raw Glycerol from Sunflower Oil Biodiesel for Growth and 1,3-Propanediol Production by *Clostridium Butyricum*. *J. Chem. Technol. Biotechnol.* **2008**, *83*, 1072–1080.
- (18) Mu, Y.; Teng, H.; Zhang, D.; Wang, W.; Xiu, Z. Microbial Production of 1,3-Propanediol by *Klebsiella Pneumoniae* using Crude Glycerol from Biodiesel Preparations. *Biotechnol. Lett.* **2006**, *28*, 1755–1759.
- (19) Chi, Z.; Pyle, D.; Wen, Z.; Frear, C.; Chen, S. A Laboratory Study of Producing Docosahexaenoic Acid from Biodiesel-Waste Glycerol by Microalgal Fermentation. *Process Biochem.* **2007**, *42*, 1537–1545.
- (20) Pyle, D. J.; Garcia, R. A.; Wen, Z. Producing Docosahexaenoic Acid (DHA)-Rich Algae from Biodiesel-Derived Crude Glycerol: Effects of Impurities on DHA Production and Algal Biomass Composition. *J. Agric. Food Chem.* **2008**, *56*, 3933–3939.
- (21) Sabourin-Provost, G.; Hallenbeck, P. C. High Yield Conversion of a Crude Glycerol Fraction from Biodiesel Production to Hydrogen by Photofermentation. *Bioresour. Technol.* **2009**, *100*, 3513–3517.
- (22) Fountoulakis, M. S.; Manios, T. Enhanced Methane and Hydrogen Production from Municipal Solid Waste and Agro-Industrial by-Products Co-Digested with Crude Glycerol. *Bioresour. Technol.* **2009**, *100*, 3043–3047.
- (23) Xiu, S.; Shahbazi, A.; Shirley, V. B.; Wang, L. Swine manure/ Crude Glycerol Co-Liquefaction: Physical Properties and Chemical Analysis of Bio-Oil Product. *Bioresour. Technol.* **2011**, *102*, 1928–1932.
- (24) Xiu, S.; Shahbazi, A.; Wallace, C. W.; Wang, L.; Cheng, D. Enhanced Bio-oil Production from Swine Manure Co-liquefaction with Crude Glycerol. *Energy Convers. Manage.* **2011**, *52*, 1004–1009.
- (25) Gan, J.; Yuan, W.; Nelson, N. O.; Agudelo, S. C. Hydrothermal Conversion of Corn Cobs and Crude Glycerol. *Biol. Eng.* **2010**, *2*, 197–210.
- (26) Mothes, G.; Schnorpfeil, C.; Ackermann, J. Production of PHB from Crude Glycerol. *Eng. Life Sci.* **2007**, *7*, 475–479.
- (27) Dobroth, Z. T.; Hu, S.; Coats, E. R.; McDonald, A. G. Polyhydroxybutyrate Synthesis on Biodiesel Wastewater using Mixed Microbial Consortia. *Bioresour. Technol.* **2011**, *102*, 3352–3359.
- (28) Manosak, R.; Limpattayanate, S.; Hunsom, M. Sequential-Refining of Crude Glycerol Derived from Waste used-Oil Methyl Ester Plant Via a Combined Process of Chemical and Adsorption. *Fuel Process. Technol.* **2011**, *92*, 92–99.
- (29) Hansen, C. F.; Hernandez, A.; Mullan, B. P.; Moore, K.; Trezona-Murray, M.; King, R. H.; Pluske, J. R. A Chemical Analysis of Samples of Crude Glycerol from the Production of Biodiesel in Australia, and the Effects of Feeding Crude Glycerol to Growing-Finishing Pigs on Performance, Plasma Metabolites and Meat Quality at Slaughter. *Anim. Product. Sci.* **2009**, *49*, 154–161.
- (30) Xiu, S.; Shahbazi, A.; Shirley, V.; Mims, M. R.; Wallace, C. W. Effectiveness and Mechanisms of Crude Glycerol on the Biofuel Production from Swine Manure through Hydrothermal Pyrolysis. *J. Anal. Appl. Pyrolysis* **2010**, *87*, 194–198.
- (31) *Standard Test Methods for Polyurethane Raw Materials: Determination of Viscosity of Polyols*; ASTM D4878-08; ASTM International: West Conshohocken, PA, 2008.
- (32) *Soap in Oil Titrimetric Method*; AOCS Recommended Practice Cc 17–95; AOCS: Urbana, IL, 1997.
- (33) *Standard Test Methods for Polyurethane Raw Materials: Determination of Acid and Alkalinity Numbers of Polyols*; ASTM D4662-08; ASTM International: West Conshohocken, PA, 2008.
- (34) *Moisture, Karl Fischer Volumetric Method*; AOCS Official Method Ea 8-58; AOCS: Urbana, IL, 1997.
- (35) *Total, Free and Combined Glycerol Iodometric-Periodic Acid Method*; AOCS Official Method Ca 14-56; AOCS: Urbana, IL, 1997.
- (36) *Total Fatty Acids, Oxidized Fatty Acids, Wet Extraction Method*; AOCS Official Method G 3-53; AOCS: Urbana, IL, 1997.
- (37) *Standard Test Method for Determination of Total Monoglyceride, Total Diglyceride, Total Triglyceride, and Free and Total Glycerin in B-100 Biodiesel Methyl Esters by Gas Chromatography*; ASTM D6584-10a; ASTM International: West Conshohocken, PA, 2010.
- (38) *Free Fatty Acids*; AOCS Official Method Ca 5a-40; AOCS: Urbana, IL, 1997.
- (39) Petrovic, Z. S. Polyurethanes from Vegetable Oils. *Polym. Rev.* **2008**, *48*, 109–155.