Monoglyceride and monoglyceride derivatives from glycerol generated in catfish based biodiesel production process

Bui Thi Buu Hue¹, Luong Thi Phuong Hong¹, Nguyen Thi Ngoc Phuoc¹, Phan Tien Si¹, Takeo Matsubara², Yoshiak Kitaya³ and Ryosuke Endo³
¹College of Natural Sciences, Can Tho University, Viet Nam
²Yanmar Agriculture Research Institute, Can Tho, Viet Nam
³Environmental Sciences and Technology, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Japan.

E-mail: btbhue@ctu.edu.vn

Abstract. Glycerol is a major byproduct in the biodiesel manufacturing process which causes environmental problem since glycerol cannot be released without treatment. In this study, crude glycerol was refined into a pure form by using phosphoric acid. Esterification of the purified glycerol with oleic acid afforded glycerol monooleate in 43% yield using p-toluenesulfonic acid as a catalyst. The obtained glycerol monooleate was then allowed to react with lactic acid, acetic acid or diacetyl tartaric acid to provide the corresponding lactylated, acetylated or diacetyl tartarylated glycerol monooleate, respectively, in reasonable yields. These ester derivatives of monoglycerides have been known to be the most commonly used food surfactants.

1. Introduction
Biodiesel is one of the most focused topic of study recently.¹-⁴ Glycerol (or glycerin) is a major byproduct in the biodiesel manufacturing process, representing 10% of the biodiesel production. The expanding production of biodiesel around the world has led to the generation of a huge amount of crude glycerol that has been becoming an environmental matter since glycerol cannot be released without treatment. Effective utilization of crude glycerol therefore not only to improve the economics of biodiesel production but also for ecological factor.

There are various ways for disposal and utilization of the crude glycerol generated in biodiesel production process. Crude glycerol can be refined into a pure form and then be used in food, pharmaceutical, or cosmetics industries. This use, however, is too expensive to be performed especially for small scale biodiesel producers. Combustion, composting, animal-feeding, thermo-chemical conversions, and biological conversion methods for glycerol usage and disposal have all been proposed.⁵-⁷ However, these methods were found not to be economical and useful. Converting crude glycerol into valued-added products such as monoglycerides and their ester derivatives by chemical methods is an attractive option to transform this biomass derived compound into fine chemicals.

Monoglycerides with saturated or unsaturated fatty acids are by far the most commonly used food surfactants.⁸-¹⁰ The first use of monoglycerides on an industrial scale was, more than 50 years ago, for making margarine where they emulsify the water phase in oil and fat phase. They are now currently included in low-calorie spreads, peanut butter, ice cream to control their texture, starch-base food (macaroni, noodles, potato products...) and baking industry. Monoglycerides are used also as raw
materials for making more lipophilic or more hydrophilic molecules utilized in cosmetics. Esters are
made with acetic, lactic, succinic and citric acids to emulsify commercial products. This paper presents
our study results on the conversion of crude glycerol from biodiesel production process into three
valued-added monoglyceride esters through chemical methods.

2. Materials and Method
Chemicals was purchased from Merck and used as received without further purification. Catfish fat was
supplied by Tra Noc industry zone, Can Tho city. \(^1\)H, \(^13\)C-NMR, DEPT spectra were recorded on an
Advance 500 Bruker. TCL was performed on silica gel 60 F254 (0.2 mm, Merck). Flash column
chromatography was carried out on silica gel Merck 60 (0.040 - 0.063 mm).

2.1 Purification of glycerol
As catfish fat used in this study had high content of free fatty acids (AV=10.23 mg KOH/g), an
acid-catalyzed pretreatment therefore was required. The objective of this stage was to reduce the acid value
of catfish fat to below about 5 mg KOH/g before going to a base induced transesterification stage.
Important variables could affect the esterification process were methanol content, catalyst loading and
reaction time. The results showed that the acid value of 1.6 mg KOH/g could be obtained by employing
the esterification conditions as follows: the temperature of 65 °C, methanol content of 35% (by the
weight of the oil), catalyst amount (by the weight of the oil) of 1% (H\(_2\)SO\(_4\)), reaction time of 1.5 hours
and agitation speed of 600 rpm (rounds per minute). The pretreated catfish fat was then subjected to the
second base catalysed transesterification stage. The effects of three reaction variables namely methanol
content, catalyst loading and reaction time on the yield of biodiesel during alkali-catalyzed
transesterification were evaluated by using RSM. The maximum biodiesel conversion yield from catfish
fat, as predicted by quadratic polynomial model, was established to be 94.12% under the optimum
reaction conditions as follows: methanol content of 20.46%, catalyst loading of 1.40% and the reaction
time of 147.20 minutes. At the end of the reaction, the mixture was separated into two layers. The
glycerin layer was fallen down to the bottom and the methyl ester (biodiesel) floated to the top. The
system was allowed to stand for about an hour and the glycerin layer was then drained off. Acidification
of the crude glycerol by concentrated solution of phosphoric acid until pH = 6-7. The resulting mixture
was allowed to stand for 24 hours and phosphate salt was filtrated. The filtrate was separated into two
layers with the crude glycerol was occupied at the bottom and then drained off, dried by anhydrous
MgSO\(_4\) and excess methanol was evaporated under reduced pressure to obtain the desired glycerol.

2.2 Preparation of glycerol monooleate (3)
A mixture of oleic acid (70.615 g – 0.25 mol), glycerol (46.045 g – 0.5 mol) and \(p\)-TsOH (1.06 g – 1.5
w% with respect to oleic acid) was stirred at 140°C for 90 minutes. The obtained mixture was neutralized
by aqueous saturated solution of NaHCO\(_3\), extracted with EtOAc. The organic layer was washed with
water until neutral, saturated solution of NaCl, dried over Na\(_2\)SO\(_4\) and solvent was evaporated under
reduced pressure to obtain 63.902 g of crude product. Purification of 0.196 g of crude product by column
chromatography to afford glycerol monooleate (0.079 g - 29% yield, \(R_f = 0.22\), PE : EtOAc = 1:1) as a
bright yellow liquid. The structure of the desired product was confirmed by \(^1\)H-NMR and \(^13\)C-NMR.

**Glycerol monooleate (3):** \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 5.37-5.32 (m, 2H, CH=CH); 4.22-4.10
(m, 2H, CH\(_2\)OCO); 3.95-3.83 (m, 1H, CHOH); 3.73-3.58 (m, 2H, CH\(_2\)OH); 2.37- 2.34 (m, 2H,
CH\(_2\)COO); 0.88 (m, 3H, CH\(_3\)). \(^13\)C-NMR (250 MHz, CDCl\(_3\)): \(\delta\) 174.3 (C=O); 130.0-129.7 (CH=CH);
70.3 (CHOH); 65.2-63.3 (CH\(_2\)); 34.1 (CH\(_2\)COO); 27.2 (2CH\(_2\)-CH); 14.1 (CH\(_3\)).

**Glycerol dioleate (4):** \(^1\)H-NMR (500 MHz, CDCl\(_3\)): \(\delta\) 5.37-5.31 (m, 2H, CH=CH); 4.19 (m, 1H,
CHOH); 4.17-4.06 (m, 2H, CH\(_2\)OCO); 2.05 (m, 1H, OH); 0.90-0.86 (m, 3H, CH\(_3\)).
2.3 Preparation of lactylated glycerol monooleate (LGO) (6a)

A mixture of glycerol monooleate (3) (2.113 g – 0.0024 mol) and lactic acid (0.45 g – 0.005 mol) was stirred at 150 °C for 4 hours. The excess lactic acid was separated. The organic layer was washed with water, saturated solution of NaCl, dried over Na₂SO₄ and solvent was evaporated to obtain 1.452 g of crude product. Purification by column chromatography afforded the desired lactylated monoglyceride (5) as a brown yellow liquid (0.726 g – 70.14% yield, R₂ = 0.429, PE:EtOAc = 2:3).

1H-NMR (500 MHz, CDCl₃): δ (ppm) 5.38-5.32 (m, 2H, CH=CH); 4.36-4.31 (m, 1H, CH(OH)); 4.30-4.19 (m, 4H, CH₂-OCO); 4.17-4.12 (m, 1H, COCH(OH)CH₃); 2.35 (t, 2H, COCH₂); 2.06-1.97 (m, 4H, 2CH₂-CH₂); 1.61-1.5 (m, 2H, COCH₂CH₂); 1.45 (d, 3H, CH(OH)CH₃); 1.42-1.26 (m, 24H, CH₂); 0.88 (t, 3H, CH₃).

13C-NMR (250 MHz, CDCl₃) δ (ppm) 171.1 (2COOH); 169.7 (2CO, 70.4 (2CH), 20.7 (2CH₂)); 65.95 (CH); 34.1 (CH₂-OCO); 27.2 (CH₂-CH); 16.81 (CH(OH)CH₃); 14.1 (CH₃).

2.4 Preparation of acetylated glycerol monooleate (6b)

A mixture of glycerol monooleate (2.113 g – 0.0024 mol) and acetic anhydride (0.128 g – 0.00125 mol) was stirred at 140°C for 30 minutes. The resulting mixture was extracted with EtOAc. The organic layer was washed with water, saturated NaCl, dried over Na₂SO₄, and solvent was evaporated under reduced pressure to afford 1.834 g of crude product. Purification by column chromatography (PE:EtOAc = 7:1) afforded acetylated glycerol monooleate (0.728 g – 73%, R₂ = 0.474, PE:EtOAc = 2:1).

1H-NMR (500 MHz, CDCl₃): δ (ppm) 5.37-5.32 (m, 2H, CH=CH); 4.34-4.30 (m, 1H, CH(OH)); 4.26-4.09 (m, 4H, CH₂-OCO); 2.37-2.34 (m, 2H, COCH₂); 2.10 (s, 3H, COCH₃); 2.08-1.97 (m, 4H, 2CH₂CH); 1.65-1.57 (m, 2H, COCH₂CH₂); 1.31-1.26 (m, 20H, CH₂); 0.90-0.86 (m, 3H, CH₃).

13C-NMR (250 MHz, CDCl₃) δ (ppm) 174.3 (C=O); 130.0 and 129.7 (CH); 68.34 (CH(OH)); 65.27 and 65.04 (CH₂OCO); 34.1; 31.9; 29.69; 29.8; 29.3; 29.5; 29.6; 29.3; 29.1; 29.5; 24.9; 22.7 (CH₂); 20.8; 14.1 (CH₃).

2.5 Preparation of diacytartaric tartaric monoglyceride (DATEM) (9)

A mixture of tartaric acid (15.009 g – 0.1 mol), acetic anhydride (51 g – 0.5 mol) and p-TsOH (0.750 g – 5%) in a 250 mL ground bottom flask was stirred at 90°C for 90 minutes under nitrogen atmosphere. Petroleum ether (50 mL) was then added and the pure product diacyltartaric acid (8) was recrystallized as a white crystalline solid in 83% yield (R₂ = 0.43, MeOH).

1H-NMR (500 MHz, CDCl₃): δ 9.09 (2H, 2COOH); 5.76 (1H, CH; 2.19-2.10 (6H, 2CH₃). 13C-NMR (250 MHz, CDCl₃): δ 177.7 (2COOH); 171.1-169.7 (2CO, 70.4 (2CH); 20.7-20.3 (2CH₃). A mixture of diacyltartrate (8) (7.02 g – 0.03 mol) and 26.5 g of crude monoglyceride (containing 0.03 mol of pure glycerol monooleate (3)) and p-TsOH (0.534 g – 5%, w% with respect to glycerol monooleate) in a 100 mL round bottom flask under nitrogen was stirred at 140°C for 60 minutes. Aqueous saturated solution of NaHCO₃ was then added (50 mL). The resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water until neutral, then with saturated aqueous solution of NaCl, dried with Na₂SO₄ and solvent was then evaporated under reduced pressure to obtain 25.387 g crude product. Purification by column chromatography (PE : EtOAc = 10:1) afforded diacyltartaric monoglyceride (9) (8.38 g – 0.015 mol – 49% yield).

1H-NMR (500 MHz, CDCl₃): δ 5.38 (d, 1H, OCHCOOH); 5.35-5.33 (d, 1H, OCHCOO); 5.27-5.25 (2H, CH=CH); 4.31-4.26 (1H, CHOH); 4.16-4.13 (2H, CH:OCO); 2.33-2.29 (2H, CH:COO); 2.07-1.95 (2H, CH₂-CH); 0.89-0.83 (3H, CH₃). 13C-NMR (250 MHz, CDCl₃): δ 173.3 – 170.5 (5C=O); 130.0-129.7...
(2CH); 85.8 (CHOOC); 69.2-68.8 (CHOH); 62.4-62.1 (CH\textsubscript{2}OOC); 34.2-21.9 (14CH\textsubscript{2}); 20.7 (2CH\textsubscript{3}COO); 14.1 (CH\textsubscript{3}).

3. Results and Discussion

3.1 Purification of crude glycerol

The crude glycerol derived from alkali-catalyzed transesterification usually has a dark brown color with a high pH (11-12). Methanol and free fatty acids (soaps) are the two major impurities contained in crude glycerol\textsuperscript{11,12}. The existence of methanol is due to the fact that biodiesel producers use excess methanol to drive the chemical transesterification to completion, and do not recover all methanol. The soaps, which are soluble in the glycerol layer, originate from a reaction between the free fatty acids present in the initial feedstock and the catalyst (base). In addition, the crude glycerol produced from biodiesel production process also contains excess base. Treatment of this crude glycerol with an acid like concentrated phosphoric acid will help convert soap back to free fatty acid and excess alkaline into phosphate salts (Figure 1). In this study, the added phosphoric acid was found to vary depending on the starting material and the technique used for biodiesel production. Therefore, it is very important to determine the best pH value for the process. The best pH value was found to be 7-8. The obtained solution was separated into three phases: the free fatty acids rose to the surface, glycerol and excess methanol from biodiesel production process occupied the middle and phosphate salts were precipitated on the bottom. The salts were filtrated and the mixture of glycerol and methanol was separated from the fatty acid layer. Evaporation of methanol recovered glycerol which could be used directly for the next step without any further purification.

![Figure 1. Purification of glycerol from biodiesel production process](image)

3.2 Preparation of glycerol monooleate

Monoglycerides and their derivatives can be obtained by the direct esterification of glycerol with carboxylic acids or by the transesterification of glycerol with carboxylic methyl esters or with
triglycerides. In this study the purified glycerol was treated with oleic acid to form glycerol monooleate. The reaction made use of para-toluenesulfonic acid (p-TsOH) as the catalyst (Scheme 1). Four reaction parameters were studied including the molar ratio between glycerol and oleic acid, the amount of the used catalyst, the reaction time and temperature. The best reaction conditions as follows: the molar ratio of oleic acid : glycerol = 1:2; the catalyst percent: 1.5 % p-TsOH (w % with respect to oleic acid); reaction temperature: 140°C and reaction time: 90 minutes. Under these conditions, the product was obtained as a light yellow liquid which contained 29% of glycerol monooleate (3) (R_f = 0.22, PE : EtOAc = 1:1) together with about 10% of glycerol dioleate (determined by column chromatography). This mixture was used as the starting material for the next step without further purification.

Scheme 1. Synthesis of glycerol monooleate

3.3 Preparation of ester derivatives of glycerol monooleate
For the formation of ester derivatives, the prepared crude glycerol monooleate (3) (containing 29% of pure glycerol monooleate (3)) was then reacted with lactic acid or acetic anhydride to afford lactylated glycerol monooleate (LGM) (6a) and acetylated glycerol monooleate (AGM) (6b), respectively (Scheme 2).
Scheme 2. Synthesis of lactylated (LGM) and acetylated glycerol monooleate (AGM)

Diacetyl tartaric ester monoglycerides (DATEM) is a known emulsifier often used in food processing industries. The synthesis of tartaric monoglyceride is presented in Scheme 3. The synthesis process started with the protection of the dihydroxy groups of tartaric acid using acetic anhydride as the acetylation agent and p-TsOH as the catalyst. The synthetic reaction conditions are as follows: the molar ratio of tartaric acid : acetic anhydride = 1:5; making use of 5% of p-TsOH (w%, with respect to tartaric acid) at 90°C for 60 minutes. For the formation of diacetyl tartarate ester monoglyceride (9), diacetyl tartaric acid (8) was treated with crude glycerol monooleate (containing 40.3% of pure glycerol monooleate 3) using p-TsOH as the catalyst. The reaction conditions as follow: the molar ratio of glycerol monooleate (3): diacetyl tartaric acid (8) = 1:1; reation temperature: 140°C; reaction time: 60 minutes; catalyst p-TsOH: 5% (with respect to glycerol monooleate (3)). Under these conditions, the desired diacetyl tartaric ester monoglyceride (9) was isolated in 49% yield (purification by column chromatography, PE : EtOAc = 10:1) as a brown solid.

Scheme 3. Synthesis of diacetyl tartaric ester monooleate (DATEM)

It is well known that lipid-based emulsifiers are versatile and functional ingredients for many types of cosmetic formulations. They combine well with most emollients and other emulsifiers to create formulations that are pleasing to the touch and have a good shelf life.
4. Conclusions
Glycerol monooleate has been prepared in reasonable yield from crude glycerol generated in biodiesel production process. Further esterification of the formed glycerol monooleate with lactic acid, diacetyltartaric acid or acetic anhydride to afford three ester derivatives of glycerol monooleate. These types of ester derivatives are promising candidates for food technology process. This research result contributes to effective utilization of crude glycerol that is very crucial to the commercialization and further development of biodiesel production process in practice.

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