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Biodiesel Refining and Glycerin Recovering Process of Transesterification from Tra Catfish Fat

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Abstract : Nowadays, Tra catfish fat is given attention as an appropriate material for biodiesel production in Vietnam. The aim of this work is to investigate the optimal conditions of refining biodiesel and recovering glycerin by the transesterification from Tra catfish fat using KOH catalyst. As our results, the yield of transesterification was achieved to 94.17% at 50°C for 45 min with 6:1 molar ratio of methanol to fat in the presence of 0.8% KOH catalyst, and wherein the biodiesel was refined by washing with distilled water at 70°C and dried in a microwave oven. The yield of raw glycerin recoveries from the transesterification process was 78.58%. The purity of raw glycerin was 84.14% by the conditions of neutralization state with H_3PO_4 solution (pH = 5), 70°C, and 60 min. Activated carbon (3.0 wt.%) was used for the bleaching process at 80°C for 20 min. The biodiesel was obtained in accordance with for ASTM D 6751 (biodiesel standard). The ash and water of raw glycerins were 7.32 and 8.01%, respectively, and implied that the raw glycerin is very promising candidate to be used as a raw material for textile and cosmetic industries.

Keywords : Biodiesel, Tra catfish fat, glycerin recovery, KOH catalyst, transesterification.

1. Introduction

Biodiesel is a diesel fuel originating from vegetable oil and animal fat. In recent years, constant increases in prices of crude oil, the scarcity of oil as a source of material, and the environmental pollution resulting from industrial and traffic wastes have encouraged research of biodiesel productions [1,2]. Biodiesel is a mono alkyl ester that is derived from long chain fatty acids of vegetable oil, animal fat or wasted oil, and prepared by the transesterification reaction or alcoholysis. A wide range of oil and fat has

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been studied as a material for biodiesel production, such as soya bean oil [3], rape-seed oil [4], sunflower oil [5], palm and coconut oil [6], and ect. Currently, as a matter of the energy security, researcher pays attention to inedible oil and fat such as Jatropha oil [7,8] Pongamia pinnata [9], karanja [10], and ricinus oil [11], waste or used oil [12], Tra catfish fat [13], and ect. The quality of biodiesel must be strictly tested according to the fuel standard. Physicochemical properties of the biodiesel are classified into two groups. The first group is the characteristics of diesel fuel. The second groups are chemical composition, purity (such as the acidity), water contents, free glycerin, mono-glyceride, di-glyceride, tri-glyceride, and residual. All values shows a completeness of transesterification reaction and the effect of washing and refining process on the quality of biodiesel [14,15,16,17]. The transesterification reaction using homogeneous catalyst, the product mixture is divided two layers after the synthesis process was completed, and the separated upper and lower layers are biodiesel and glycerin, respectively. The composition of biodiesel layer are methyl ester of fatty acid (FAME), methanol and catalyst, free glycerin (GL), diglyceride (DG), monoglyceride (MG), triglyceride (TG), free fatty acid, soap, and water. The lower layers are glycerin (the main of component), methanol, residual catalyst, few fatty acids such as FA, TG, DG, and MG. The purity of produce is improved by washing and refining process. The EN 14214:2003, ASTM D 6751, or DIN V 51606 standards have been applied for the diesel engine and the indices of biodiesel such as the free fatty acid, GL, DG, TG, and water contents must be controlled to minimized value. However, the cost of this process increases the total cost of production of biodiesel, thus, this is a significant impediments of the development of biodiesel production for less developed country. To solve the problem of production cost, some studies are researched solution using heterogeneous catalyst and recover glycerin process, and using recover glycerin as a raw material for other industry. On the other hand, the cost-efficient methods could be decreased by the optimum of washing and separating process, and improves the purity of biodiesel.

F. Karaosmanoglu *et al.* [18] have studied that the conditions for the washing and refining stage of biodiesel prepared from crude and neutralized rape-seed oil with methanol using NaOH catalyst. The authors compared three washing methods: hot distilled water, dissolve biodiesel phase in oil ester and then wash with hot distilled water, and neutralize with H_2SO_4. The results found that the yield of biodiesel reached 86.3% with a purity of 99%, a water content of 0.095%, and a free acidity of 0.40 when the washing and refining process was carried out with hot distilled water at 50°C and then dried with 20% heated Na_2SO_4 (relative to the volume of biodiesel). Ooi *et al.* have reported that the process of glycerin recovery from biodiesel production with palm oil and methanol using NaOH catalyst [19]. The authors used H_2SO_4 to neutralize the residual NaOH catalyst, separated fatty acid, neutralized with NaOH, and separated salt in the deposition method. After volatilization with distillation to increase the concentration of glycerin, the purity of GL was improved by dissolving the resulting glycerin with an organic solvent, filtering and distilling. The crude GL had been recovered at 51.4% with other components: 8.9% water, 25.9% non-glycerin organics, and 13.8% ash. The low pH (1-2) is determined as the best for the recovery process.

In the present work, the optimal conditions of washing and refine biodiesel process were investigated. Using Tra catfish fat as a raw material for biodiesel production, the glycerin was recovered from the biodiesel production

using KOH catalyst and methanol.

2. Experimental

2.1 Materials

Tra catfish fat (Tra fat) was supplied by An Giang Agriculture and Food Export Company (Afiex, Vietnam). The potassium hydroxide catalysts (86%), sodium hydroxide (82%), CH_3COOH, Na_2SO_4, and H_3PO_4 (China) were used in this research. Commercial grade activated carbon and methanol (90%) were used directly without purification. The gas chromatograph standards (Fluka, United Kingdom) for methyl esters were used for purity analysis. Other analytical chemicals were supplied by Merck(Germany). A domestic microwave oven (Sanyo, 1200 W) was used for drying biodiesel.

2.2 Methods

2.2.1 Transesterification of Tra fat

The KOH catalyst contents (0.8 wt %) was calculated from the total weight of fat plus methanol prior to reaction. The appropriate catalyst and methanol were placed in a 250 cm^3 two-neck flask, which was equipped with a reflux condenser. The mixture was stirred (600 rpm) for 20 min. Then the flask was immersed in a constant-temperature bath and heated up to required temperature. Following that, 0.05 mol of (42.86g) fat was added in the flask. The mixture was stirred with the same rate at 50°C for 45 min. After transesterification was completed, the excess of methanol was recovered under reduced pressure at 60°C with a rotary evaporator. The mixture was divided into two phases by a separatory funnel after 4 h. The upper separated layer was biodiesel phase and the lower layer was glycerin phase. The biodiesel phase was applied to the study of washing and refining process. The glycerin phase was used for the study of recover glycerin process.

2.2.2. Biodiesel washing and refining process

Using biodiesel phase as a raw material, the washing and refining biodiesel process were investigated. The block diagram for washing and refining process is shown in Fig. 1.

The residual KOH catalyst was removed by the neutralizing method with 2% CH_3COOH, or with 2% H_2SO_4, or washed with hot distilled water at 60°C. The mixture was rewashed with hot water and dried for 10 min in a microwave oven.

The ratio of biodiesel and distilled water was 1:1. Soap, GL, MG, DG, and TG were removed by the washing process. The distilled water at 50°C, 60°C, 70°C, and 80°C, and dried for 10 min in a microwave oven were analyzed.

The water was removed by four methods: Dried with 5% heated Na_2SO_4 (calculated by the volume of raw biodiesel) for 12 h, a vacuum rotary evaporation in at 80°C for 2 h, dried in the oven at 105°C until the weight of product was no changed, and dried for 10 min in a microwave oven.

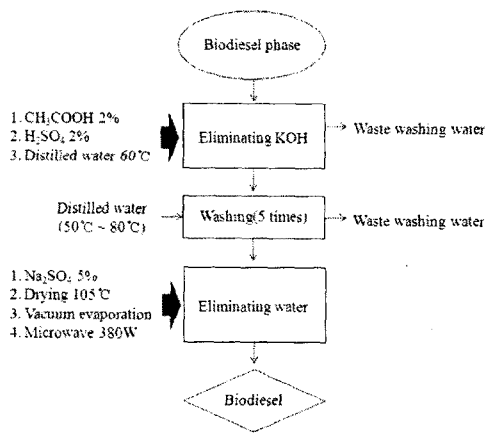

Fig. 1. Schematic diagram of the washing and refining process.

2.2.3 Glycerin recovery process

The glycerin phase was used for the research of recover process. The recover process of glycerin as showed in Fig. 2. The glycerin recovery process including: removing residual methanol, removing catalyst with excess H_3PO_4, separating salt and fat organics, bleaching with activated carbon, reneutralizing excess H_3PO_4 with NaOH, eliminating inorganic salts, and removing water.

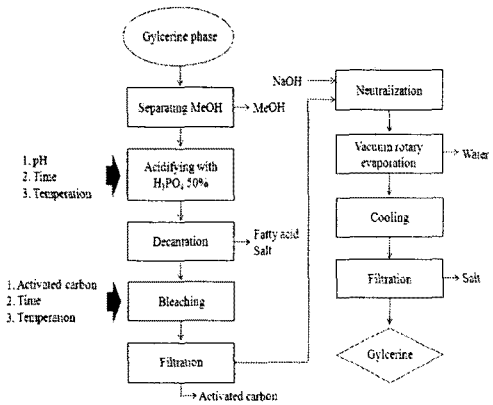

Fig. 2. Schematic diagram of the crude glycerin recovery process.

The steps of recovering glycerin were performed as follows: after eliminating methanol by a vacuum rotational evaporation, neutralize KOH catalyst by excess 50% H_3PO_4 and adjusted appropriate pH, and controlled reaction temperature and time. Five hours later, the mixture was divided into three layers by a separator funnel. The separated upper, mid and lower layers were fatty acid, raw glycerin and inorganic salt, respectively. The mixture of glycerin was bleached by activated carbon. The mixture solution (raw glycerin and organic salt) was obtained after eliminating water by a vacuum rotary evaporation. The organic salt was removed by the separation process at -3°C.

The aim of this research is to investigate the effects of pH, temperature, and time on the catalyst-neutralized stage. The bleaching stage (activated carbon contents, bleaching temperature and time) also was analyzed. In the first stage, the activated carbon contents was 3.0 wt.% (calculated by the raw glycerin contents), the temperature and time were fixed at 60°C for 20 min.

2.3 Analytical methods

2.3.1 Biodiesel

Important fuel properties of methyl ester of Tra fat were prepared in accordance with ASTM D6751 (biodiesel standard). The fatty acid compositions of Tra fat were determined by the AOAC method. The fatty acid compositions of Tra fat were determined by the GC-ISO/CD 5509:94 method. Quantitative analyses of FAME were carried out by the GC method. The analyses were performed on a Hewlett-Packard 6890 Series Gas Chromatograph System equipped with FID detector using the HP INNOWAX column (30 m x 0.53 mm x 1 μm) and helium as a carrier gas. Methyl hexanoate was used as a material standard. The split ratio was 50:1. The temperature of detector was 250°C. Temperature of oven was started at 120°C, kept for 2 min, up to 230°C (7 °C/min), and then kept this condition for 15 min. Quantitative analyses of TG, DG, MG, and GL were also performed on the same Hewlett-Packard 6890 using the DB-5HT column (30 m x 0.250 mm x 0.1 μm) and helium used as a carrier gas. Before testing by the GC, samples were reacted with MSTFA in the presence of pyridine to give the corresponding volatile trimethylsilyl derivatives of the hydroxyl group. Two internal standards were 1,2,4-butanetriol and 1,2,3-tricaproylglycerol (tricaprin). The split ratio was 5:1. Temperature of injector and detectors were 350°C and 380°C, respectively. Temperature of oven was started at 60°C and kept for 1 min, up to 180°C (15°C/min), increased to 230°C (7°C/min), up to 330°C (10 °C/min), continuously increased to 375°C

(15°C/min), and then kept this condition for 4 min.

The yield of production is defined as the weight percentage of biodiesel relative to the weight of Tra fat. The purity of biodiesel production was calculated from the area of fat catfish methyl ester over the reference area analyzed by the gas chromatograph method.

$$H(\%) = \frac{m_{biodiesel}}{m_m} \times 100\%$$

$$P(\%) = \frac{\sum A - A_{EI}}{A_{EI}} \times \frac{C_{EI} \times V_{EI}}{m} \times 100\%$$

where, A : Sum area of methyl ester peaks from $C_{14:0}$ to $C_{24:1}$

A_{EI} : Peak area of methyl hexanoate

C_{EI} (mg/ml) : Concentration of methyl hexanoate

V_{EI} (ml) : Volume of methyl hexanoate

m_m (mg) : Tra fat amount

2.3.2 Glycerin

After the transesterification reaction was completed, the glycerin contents of biodiesel phase (m_{GL1}) and glycerin phase (m_{GL2}) were analyzed in order to determine the total amount of glycerin in the conversion process. The total glycerin contents were calculated by theoretical equation. m_{GL} was a glycerin amount that was obtained by the recovery process.

The yield of the glycerin recovery process (H_{GL}):

$$H_{GL}(\%) = \frac{m_{GL}}{m_{GL1}} \times 100\%$$

The quality of the recovered glycerin was evaluated by the content of pure glycerin, pH, water, ash, and residue contents.

3. Results and Discussion

3.1 Characterizations of Tra fat

The physicochemical properties of Tra fat were 0.9081 of specific gravity(30°C), 0.3 wt.% of water contents, 4.4 of acid value, 63.4 of Iodine value, and 207 of saponification value. The unsaturated content of Tra fat was 54.7%, and the main composition of fatty acids were $C_{18:1}$ and $C_{18:2}$ as shown in Table 1. The unsaturated contents of Tra fat, palm oil (56.6%), and edible beer tallow (52.0%) were similar. However, these values were lower than those of vegetable oils, such as sunflower, rapeseed, and soybean[20,21]. Freedman *et al.* have proclaimed that fat with high acid value(4.4) is not appropriate to the transesterification reaction for the biodiesel synthesis using the alkaline catalyst, because they produce soaps by neutralizing the free fatty acids and saponificated triglyceride [22].

Table 1. The fatty acid compositions of Tra fat

Fatty acids	Content (wt %)
$C_{14:0}$	3.85
$C_{16:0}$	29.55
$C_{18:0}$	7.78
$C_{18:1}$	38.37
$C_{18:2}$	14.25
$C_{18:3}$	0.93
$C_{20:1}$	1.16
% Unsaturated fatty acid	54.70

3.2 The biodiesel washing and refining process

3.2.1 The elimination of KOH catalyst

After separating phases, the excess KOH catalyst had to be removed from the biodiesel phase. Three different methods were applied: (a) Neutralizing with 2% H_2SO_4, (b) neutralizing with 2% CH_3COOH upto pH≈7, then the resulting biodiesel was washed with

hot distilled water and slightly stirred five times at 60°C, and (c) only washing with hot distilled water five times at 60°C.

The optimum KOH elimination condition was found to be the highest yield of biodiesel (94.7%) by washing with the hot water at 60°C. Using acid-neutralized method decreased the yield of biodiesel, because the biodiesel was lost during neutralization process. The yield of biodiesel production using CH_3COOH (93.8%) as a neutralizing reagent was slightly higher than that using H_2SO_4 (92.02%).

3.2.2 The washing temperature with water

The washing temperatures with distilled water were varied as 50, 60, 70, and 80°C. The results showed that the yield of biodiesel was dependent on the washing temperature with water. The lowest yield of biodiesel(91.3%) was obtained at 50°C. The highest yield of biodiesel was 94.71 at 60°C. At this condition, the washing process could be carried out fast and simply. Nevertheless, when the washing temperatures with water were increased to 70 and 80°C, the yields of biodiesel were decreased to 92.45% and 91.89%, respectively. These result means the emulsion of fatty acids, soap and washing water might be formed at higher temperature. The emulsion produced some air bubbles at higher temperature, thus, the emulsion was easily ran out of the vessel. Even we applied the higher washing temperature with water (60°C) than Karaosmanoglu's condition[18], our yield of biodiesel production (94.71%) was higher than that of their reports (84.2%). The difference of results might be due to using different fat materials and reaction conditions.

3.2.3 Drying

After removing the KOH catalyst, residues, TG, DG, MG, and GL, biodiesel had to dry to satisfy the fuel standard. Some methods were investigated including with the hot 5% Na_2SO_4 (based on the amount of crude biodiesel). A rotary evaporator under vacuum, a dryer, and a domestic microwave oven were applied for KOH elimination. The results found that drying biodiesel in a microwave oven showed the highest yield of biodiesel (94.71%) and the higher value (94.63%) was obtained by using hot Na_2SO_4. In terms of drying, both methods of the vacuum rotary evaporation and drying under dryer gave the lower yield of biodiesel(88.8% and 93.2%, respectively) due to some loss under this condition. Particularly, after drying by a microwave, the water contents was a trace in the biodiesel production. Additionally, biodiesels produced by four different drying methods showed the similar physicochemical properties, such as the specific gravity, viscosity, acid value, total and free glycerin contents, and etc. All our results were also satisfied with the ASTM D 6751 standards (Table 2). Also, using the heated Na_2SO_4 and a rotary evaporation under vacuum did not have effect on the economy of drying biodiesel process because of more materials and complicated operations. As a result, our new method showed shorter drying time than those of the vacuum rotary evaporation and the heated Na_2SO_4 methods (8.3% in comparison with the vacuum rotary evaporation method and 1.4% in comparison with the heated Na_2SO_4 method). Since our new method was a simple method with lowest costs. it would be reduced the cost and promote the commercial biodiesel production. Finally, the best conditions for the biodiesel refining process were using the distilled water at 60°C for the washing process and then drying by a domestic microwave.

3.3 The recovery of raw glycerin

As our results, the glycerin contents of biodiesel phase and glycerin phases were 0.196% and 13.1%, respectively. The yield of

transesterification reaction was 93%. However, the main of this report is to investigate the recovery process of glycerin phase.

3.3.1 The neutralizatioin condition

3.3.1.1 pH

The fixed factors in this study were 50% H_3PO_4 at 70°C for 90 min. pH was investigated from 2 to 6. The results found that at lower pH (2 and 3) the glycerin recovery yields was obtained low value (39.51% and 40.96%, respectively). With pH=4 and pH=5, the yield of glycerin recovery was increased by 77.8% and 78.58%, respectively, and shown lower values with pH=6 (48.89%). The oxidation reaction was occurred with lower pH, using more H_3PO_4, and higher temperature (70°C). Thus, the yield of recovery process was decreased, and the glycerin colors became dark color. In addition, since excess acid needed more NaOH for the neutralization process, produced more salts, thus, the yield of glycerin recovery was decreased. At pH=6, the neutralization process was not completed, so it was more difficult to separate fatty acid and salt, and the yield of glycerin recovery was also decreased. The optimal pH for the neutralization process was found to be 5, which provided the highest values for the glycerin recovery as compared with other conditions.

3.3.1.2 Time

The fixed factors of this study were 50% H_3PO_4, pH=5, and 70°C. The investigated reaction time was 30, 60, 90, and 120 min. The result showed that the neutralization reaction was not completed and the yield of recovery was low value (55.36%) with a short time for the neutralization reaction (30 min). The yield of glycerin recovery with 90 min of recovery times (79.02%) showed the highest values as compared with other conditions. The yield of glycerin recovery was obtained low value with long time recovery process (above 90 min), one part of the product was burned, and the yield was reduced (78.58% and 73.78%). Therefore, the reaction time should be chosen 60 min for following research.

3.3.1.3 Temperature

The fixed factors in this study were 50% H_3PO_4, pH=5, and 60 min. The reaction temperature was investigated at 50, 60, 70, 80, and 90°C. Since H_3PO_4 is a moderate acid, the neutralization reaction happened more quickly during higher neutralization temperature. In particular, the glycerin recovery yields were increased from 59.49% to 78.58% with increasing temperature from 50°C to 70°C. However, the glycerin recovery yields were decreased from 78.12% to 73.44% with increasing temperature from 80°C to 90°C. With much higher temperature, one part of the product was burned, the water was evaporated, and hampered the following stages. Thus, the yield of recovery was decreased. Conclusively, the suitable temperature of neutralization reaction was found to be 70°C. The best conditions for the neutralization reaction with KOH catalyst were pH=5, 70°C of neutralization temperature, and 60 min of neutralization time. The maximum yield of glycerin recovery was 78.58%. These conditions were applied to the bleaching stage.

3.3.2 The bleaching process

The glycerin was bleached at 60°C for 20 min. The activated carbon with 1.0, 2.0, 3.0, 4.0, and 5.0 wt.% was used for this condition. The results found that with lower carbon contents (1.0 to 2.0 wt.%) the recovered glycerin was still yellowish color. The color of recovered glycerin was as same as that of pure glycerin with increasing activated carbon contents up to 3.0 wt.%. The color of recovered glycerin, and the yield

of recovered glycerin were without changed with increasing activated carbon content above 4.0 wt.%. The optimal activated carbon contents for the glycerin bleaching was be found be 3.0 wt.%. The condition of bleaching process with 80°C for 20 min were supplied the best result as compared with other conditions.

Accordingly, the glycerin recovery yields of biodiesel production was 79.69% with the conditions: Neutralized with 50% H_3PO_4, pH=5, 70°C, 60 min, and bleached with 3.0 wt.% of activated carbon (calculated by the raw biodiesel volume) at 80°C for 20 min.

3.4 The properties of refined biodiesel and recovered glycerin

The physico-chemical properties of refined biodiesel and recovered glycerin are demonstrated in Table 2. The recovered glycerin was a colorless and transparent liquid. The infrared spectrum of recovered glycerin is presented in Fig. 3.

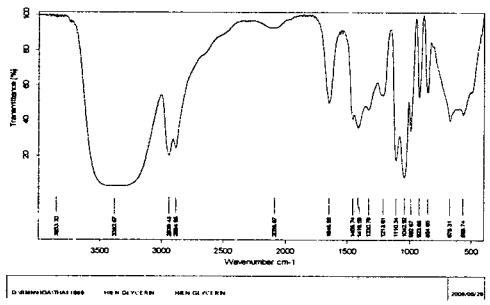

Fig. 3. The infrared spectrum of the recovered glycerin.

4. Conclusions

The technology of refining biodiesel and recovering glycerin was an important process in order to improve the biodiesel quality and to reduce the cost of biodiesel production. The biodiesel was obtained by the transesterification reaction with 6:1 molar ratio of methanol to Tra fat in the presence of 0.8 wt.% KOH catalyst at 50°C for 45 min. The yield of biodiesel production was 94.17% after washing with distilled water at 60°C and dried in a microwave oven for 10 min. The raw glycerin could be obtained 76.69% by neutralizing with excess 50% H_3PO_4 solution(pH=5) at 70°C for 60 min, and then by bleaching with activated carbon (3.0 wt.%) at 80°C for 20 min. The final raw glycerin with 8.01% of water content and 7.32% of ash contents could be applied for the soap and dye industries.

References

1. J. V. Gerpen, B. Shanks, R. Prusko, and D. Clements, Biodiesel Production Technology, Report of National Renewable Energy Laboratory (NREL/SR-510-36244), 2004.
2. H. Fukuda, A. Kondo, and H. Noda, Review Biodiesel Fuel Production by Transesterfication of Oil, *J. Biodiesel and Bioengineering*, **92**, 405 (2001).
3. W. Xie, W. X. Huang, and H. Li, Soybean oil methyl esters preparation

Table 2. The physico-chemical properties of refined biodiesel and recovered glycerin

Biodiesel	Value	Recovery glycerin	Value
Density (g/ml)	0.8674	Density (g/ml)	1.2615
Viscosity (40°C, mm/s)	4.46	Ash content (%)	7.32
Acid value (mg KOH/g)	0.47	Water content (%)	8.01
Total glycerin (%)	0.18	Pure glycerin (%)	84.18
Free glycerin (%)	0.015	-	-

using NaX zeolites loaded with KOH as a heterogeneous catalyst, *Bioresource Tech.*, **98**, 936 (2007).
4. S. Saka and D. Kusdiana, Biodiesel fuel from rapeseed oil as prepared in supercritical methanol, *Fuel*, **80**, 225 (2001).
5. A. K. Tiwari, A. Kumar, and H. Rahema, Optimisation of biodiesel production by sunflower oil transesterification, *Bioresource Tech.*, **83**, 111 (2002).
6. J. Jitputti, B. Kitiyanan, P. Rangsunvigit, K. Bunyakiat, L. Attanatho, and P. Jenvanitpanjakul, Transesterification of crude palm kernel oil and crude coconut, *Chem. Eng. J.*, **116**, 61 (2006).
7. A. K. Tiwari, A. Kumar, and H. Raheman, Biodiesel production from Jatropha oil (Jatropha curcas) with high free fatty acids: An optimized process, *Biomass and Bioenergy*, **31**, 569 (2007).
8. H. J. Berchmans and S. Hirata, Biodiesel production from crude Jatropha curcas L. seed oil with a high content of free fatty acids, *Bioresource Tech.*, **99**, 1716 (2008).
9. S. K. Karmee and A. Chadha, Preparation of biodiesel from crude oil of Pongamia pinnata, *Bioresource Tech.*, **96**, 1425 (2005).
10. P. K. Srivastava and M. Verma, Methyl ester of karanja oil as an alternative renewable source energy, *Fuel*, **87**, 1673 (2008).
11. V. Scholza and J. N. Silva, Prospects and risks of the use of castor oil as a fuel, *Biomass and Bioenergy*, **32**, 95 (2008).
12. M. Canakci, The potential of restaurant waste lipids as biodiesel feedstocks, *Bioresource Tech.*, **98**, 183 (2007).
13. L. T. T. Huong, P. M. Tan, T. T. V. Hoa, and S. Lee, Biodiesel Production From Fat of Tra Catfish and Basa Catfish, *J. of The Korean Oil Chemists' Society*, **25**, 299 (2008).
14. G. Knothe, Analytical method used in the production and fuel quality assessment of biodiesel, *Ame. Soc. of Agricultural Engineers*, **44**, 193 (2001).
15. G. Knothe, Analyzing biodiesel: Standards and other methods, *J. Am. Oil Chemist's Soc.*, **83**, (10), 823 (2006).
16. L. C. Meher, D. V. Sagar, and S. N. Naik, Technical aspects of biodiesel production by transesterification a review. *Renewable and Sustainable Energy Reviews*, **10**, 248 (2006).
17. J. V. Gerpen, B. Shanks, D. Clements, G. Knothe, and R. Pruszko, *Biodiesel Analytical Methods*, Subconstractor report, Golden, Colorado: National Renewable Energy Laboratory, 6 (2004).
18. F. Karaosmanoglu, K. B. Cigizoglu, M. Tuter, and S. Ertekin, Investigation of the refining step of biodiesel production, *Energy & Fuels*, **10**, 890 (1996).
19. T. L. Ooi, K. C. Yong, K. Dzulkefly, W. M. Z. Wanyunus, and A. H. Haimah, Crude glycerin recovery from glycerol residue waste from a palm kernel oil methyl ester plant, *J. Oil Palm Research*, **13**, 16 (2001).
20. R. Sarin, M. Sharma, S. Sinharay, and R.K. Malhotra, Jatropha-Palm biodiesel blends: An optimum mix for Asia, *Fuel*, **86**, 1365 (2007).
21. D. Zheng and M. A. Hanna, Preparation and properties of methyl esters of beef tallow, *Bioresource Techn.*, **57**, 139 (1996).
22. B. Freedman, E. H. Pryde, and T. L. Mounts, Variables affecting the yields of fatty esters, *J. Am. Oil Chemist's Soc.*, **61**, 1638 (1984).