

## Fractionation and Characterization of Insolubles Formed in Palm Oil Biodiesel

Vladimir Plata<sup>a,\*</sup>, Magda Serrano<sup>a</sup>, Karoll Tiria<sup>a</sup>, Arnold R. Romero Bohórquez<sup>b</sup>, Viatcheslav Kafarov<sup>a</sup>, Paola Gauthier-Maradei<sup>c</sup>, Edgar Castillo<sup>d</sup>

<sup>a</sup>Research Center for Sustainable Development in Industry and Energy, Industrial University of Santander, Carrera 27 calle 9, Bucaramanga, Colombia

<sup>b</sup>Organic and Biomolecular Chemistry Laboratory, Industrial University of Santander, Carrera 27 calle 9, Bucaramanga, Colombia

<sup>c</sup>INTERFASE, Industrial University of Santander, Carrera 27 calle 9, Bucaramanga

<sup>d</sup>Colombian Petroleum Institute ICP-Ecopetrol, Piedecuesta, Colombia  
vladimirplata@gmail.com

During the last decade, biodiesel has emerged as a promising alternative to diesel. However, the sedimentation of some insoluble material in pure biodiesel has become a concern for its producers. This material easily drops out of solution, settles on the bottom of biodiesel storage tanks and forms deposits on vehicle fuel filters. In this work, the insoluble material that forms in Colombian palm oil biodiesel was characterized for the first time. Non-expensive and easy to use Thin Layer Chromatography and Fourier Transform Infrared Spectroscopy were used as analytical techniques. The insoluble material was found not to be composed by free steryl glucosides exclusively. Acylated steryl glucosides appeared to be part of the insoluble material as well. This finding should lead to rethink the strategies that have been developed to prevent the formation of haze in biodiesel so far, as they have been mainly focused on the removal of free steryl glucosides from biodiesel.

### 1. Introduction

During the last decade, biodiesel has emerged as a promising alternative to traditional diesel. It offers many important advantages such as higher cetane number, lower emissions of most regulated species and biodegradability (Plata et al. 2010). In Colombia, the local government has implemented a Biofuel National Program since 2008. This program was designed in order to develop the agricultural sector, generate permanent jobs, improve the air quality and replace illicit crops. These facts have made Colombia one of the leaders of biofuel production in Latin America (Janssen and Rutz, 2011). A mandatory B10 blend is currently used in almost all the country, and the local government has planned to increase the percentage of blending in the next years. However, the sedimentation of insoluble material in pure biodiesel has become a concern. This material can appear at temperature above the biodiesel cloud point or even if biodiesel stands at room temperature for extended periods. It settles on the bottom of biodiesel storage tanks and forms deposits on diesel engine injectors (Plata et al. 2012).

Research made to identify the nature of haze has revealed that it may be a mixture of several compounds. Tang et al. (2008a) detected free and acylated sterols, monoglycerides (MG) and free steryl glucosides (FSG) in the insoluble material isolated from cotton seed oil biodiesel. Lin et al. (2011) identified soaps in the sediments collected from the bottom of storage tanks filled with canola oil biodiesel. Research has also revealed that the composition of the haze may depend on the feedstock oil and technologies used to process it (refining and transesterification). Tang et al. (2008b) examined the insoluble material isolated from palm oil biodiesel and registered only signals attributable to MG. On the contrary, Van Hoed et al. (2008) found that FSG were the major components of the cake collected after the filtration of biodiesel between storage tanks in palm oil biodiesel facilities. Similar results were reported by Bondioli et al. [6].

Until now, most of the research has focused on the characterization of haze isolated from biodiesel produced in Europe and the USA. To the best of our knowledge, the insoluble material that forms in Colombian palm oil biodiesel has not been characterized so far. In this regard, a sample of haze isolated from biodiesel produced in Ecodiesel Colombia S.A. (one of the largest providers of palm oil biodiesel in Colombia) was characterized in this paper. It was first purified by successive washes with hexane, then fractionated by column chromatography and finally characterized. Thin layer chromatography (TLC) and Fourier transform infrared spectroscopy (FTIR) were used as analytical techniques. As a result, the haze was found to be mainly composed by MG. FSG were also identified. This finding should lead to rethink the strategies that have been developed to prevent the formation of haze in biodiesel so far since they have been mainly focused on the removal of FSG from biodiesel.

## **2. Materials and methods**

### **2.1 Preparation**

The haze was taken from turbid biodiesel stored in plastic containers for a month at about 20 °C. 1 g of sample was washed with 10 ml of hexane in order to remove the residual biodiesel contained therein. The slurry formed was stirred in a Heidolph Unimax 2010 shaker for 1 min at 300 rpm and then centrifuged for 15 min at 2,100 rpm and 25 ° C using a Hettich Universal 320R centrifuge. The supernatant was removed after centrifugation and the solid extracted was placed in an oven at 80 °C for 30 min and then macerated. This solid was denominated Hz.

### **2.2 Fractionation**

Fractionation of the haze was performed by column chromatography using a 0.7 x 0.3 m glass column packed with approximately 55 g of silica gel 60 (0.063-0.200 mm, Merck ref no. 1.07734.1000) previously activated in a oven at 80 °C for 4 h. The silica gel was rinsed with three portions of hexane and the solvent was drained off until it reached the top of the silica. 1 g of sample was dissolved in chloroform/methanol (85:15 vol/vol), and silica gel was added to this solution. The slurry formed was rotoevaporated until dryness, and the solid obtained was introduced onto the column. The column was then washed successively with hexane/ethyl acetate, pure ethyl acetate, ethyl acetate/methanol and pure methanol. The fractionation was monitored by TLC, and the fractions collected were rotoevaporated until dryness and weighted.

### **2.3 Characterization**

Two simple TLC evaluations were first performed on TLC silica gel 60 F254 plates purchased from MERCK (ref. no. 1.05715.0001). Samples of the fractions collected and reference standards of FSG and monopalmitin were applied on two different TLC plates. The plates were developed with hexane/ethyl acetate (1:1 vol/vol) mixture in one evaluation and ethyl acetate/methanol (10:1 vol/vol) mixture in the other evaluation. Characterization by FTIR was performed using a Bruker Tensor 27 spectrometer equipped with an attenuated total reflectance accessory (with diamond crystal). All the spectra were obtained in the wave number range of 4,000-650  $\text{cm}^{-1}$ , using an average of 16 scans with a spectral resolution of 4  $\text{cm}^{-1}$  and air as the background. All chemical reagents used were analytical grade. The standard of FSG was purchased from Matreya LLC. The standard of monopalmitin was purchased from Sigma Aldrich Inc. Detection was carried out under iodine.

## **3. Results and discussion**

### **3.1 Preparation and fractionation**

A "washing and drying" yield, defined as the ratio between the weight of solid and the weight of unwashed haze, higher than 15 wt% was not achieved. This result was consistent with that reported by Moreau et al (2008), who analyzed samples of haze isolated from a variety of locations in the soybean oil biodiesel supply chain. These authors found that most of the samples were mainly composed by fatty acid esters. They also encountered that the amount of FSG ranged between 0 and 68 %w/w.

Two fractions were obtained during the fractionation. The first fraction, denominated F1Hz, was obtained after washing the column successively with hexane/ethyl acetate (5:1, 1:1, 1:10 vol/vol), pure ethyl acetate and ethyl acetate/methanol (10:1 vol/vol). The second fraction, denominated F2Hz, was obtained after using ethyl acetate/methanol (10:1, 1:1 vol/vol) and pure methanol. The amount of solid collected was 0.7668 mg for F1Hz and 0.1225 mg for F2Hz.

### 3.2 Characterization

Hz, F1Hz and the monopalmitin standard marked a single spot with a retention factor (Rf) of about 0.35 when the TLC plate was developed with the hexane/ethyl acetate mixture (see Figure 1, number 1, 2 and 5 on the left TLC plate). On the contrary, F2Hz and FSG standard did not mark any spots. When the TLC was developed with the ethyl acetate/ methanol mixture, the spots previously marked by both F1Hz and monopalmitin standard increased their Rf to nearly 0.91 (number 1, 2 and 5 on the right TLC plate). However, in this case, both F2Hz and FSG standard marked a single spot with Rf of about 0.63 (number 3, 4 and 6 on the same TLC plate). These results suggested that the haze was composed by a mixture of MG, which is consistent with the report of Tang et al. (2008b), and also by FSG, which is consistent with the finding of Bondioli et al. (2008) and Van Hoed et al. (2008). Monopalmitin was selected because saturated monoglycerides have been reported as the main glyceride compounds capable of forming haze in biodiesel (Farahani et al. 2011), and palm oil biodiesel is mainly composed by palmitic acid (about 45 %w/w (Prada et al. 2011)).

Figure 2 shows the FTIR spectrum obtained for the monopalmitin standard. A series of strong bands, which arises from twisting and wagging bending of  $-\text{CH}_2$ , was registered in the region usually referred as the "finger-print" ( $1,300\text{--}900\text{ cm}^{-1}$ ). A doublet for stretching of  $-\text{OH}$ , sharp absorption peaks attributable to asymmetric and symmetric stretching of  $-\text{CH}_2$ , a strong band that arises from stretching of  $-\text{C}=\text{O}$  in carboxyl and a sharp absorption peak attributable to rocking bending of  $-\text{CH}_2$  were also registered in the spectrum at  $3,299$ ,  $2,916$ ,  $2,850$ ,  $1,730$  and  $720\text{ cm}^{-1}$ . Similar strong absorption peaks were registered on the spectrum of F1Hz (see Figure 3). All these signals are characteristic of glycerides (Tang et al. 2008a).

Figure 4 shows the FTIR spectrum obtained for the FSG standard. This spectrum displayed a broad absorption band instead of a doublet for stretching of  $-\text{OH}$  at  $3,373\text{ cm}^{-1}$ , less sharp absorption peaks that arise from asymmetric and symmetric stretching of  $-\text{CH}_2$  at  $2,932$  and  $2868\text{ cm}^{-1}$ , respectively, and signals attributable to asymmetric and symmetric stretching of  $\text{C}-\text{O}-\text{C}$  and asymmetric stretching of  $\text{O}-\text{C}-\text{C}$  in glucoside moiety at  $1,070$ ,  $1018$  and  $1,165\text{ cm}^{-1}$ , respectively. Similar absorption peaks were registered on the spectrum of F2Hz (see Figure 5). All these signals are characteristic of steryl glucosides (Bondioli et al. 2008; Van Hoed et al. 2008).

An additional absorption peak registered on the F2Hz spectrum at  $1,733\text{ cm}^{-1}$  was attributed to the presence of ASG. In a previous work, Lacoste et al. (2009) reported that these compounds can coelute with the FSG present in soybean oil and palm oil when these oils were purified by column chromatography.

### 4. Conclusions

The insoluble material that forms in Colombian palm oil biodiesel, characterized in this work for the first time, consists in a mixture of monoglycerides and free steryl glucosides. More than three quarters of its mass consisted in monoglycerides, which are probably mostly monopalmitin. Acylated steryl glucosides appeared to be part of the haze as well, but more research is needed to confirm this preliminary finding. In any case, these results should lead to rethink the strategies that have been developed to prevent the formation of haze in biodiesel so far, as they have been mainly focused on the removal of free steryl glucosides from biodiesel.

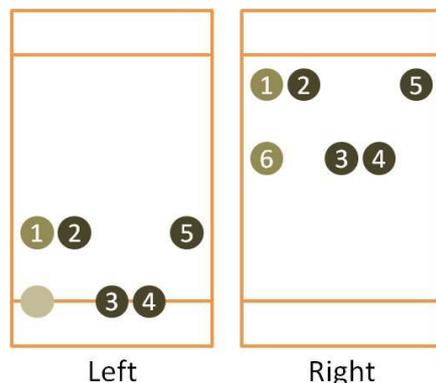


Figure 1: Scheme of TLC evaluations. Left TLC plate: developed with hexane/ethyl acetate. Right TLC plate: developed with ethyl acetate/ methanol. 1. Hz1 first spot, 2. F1Hz, 3. F2Hz, 4. FSG standard, 5. Monopalmitin standard, 6. Hz1 second spot

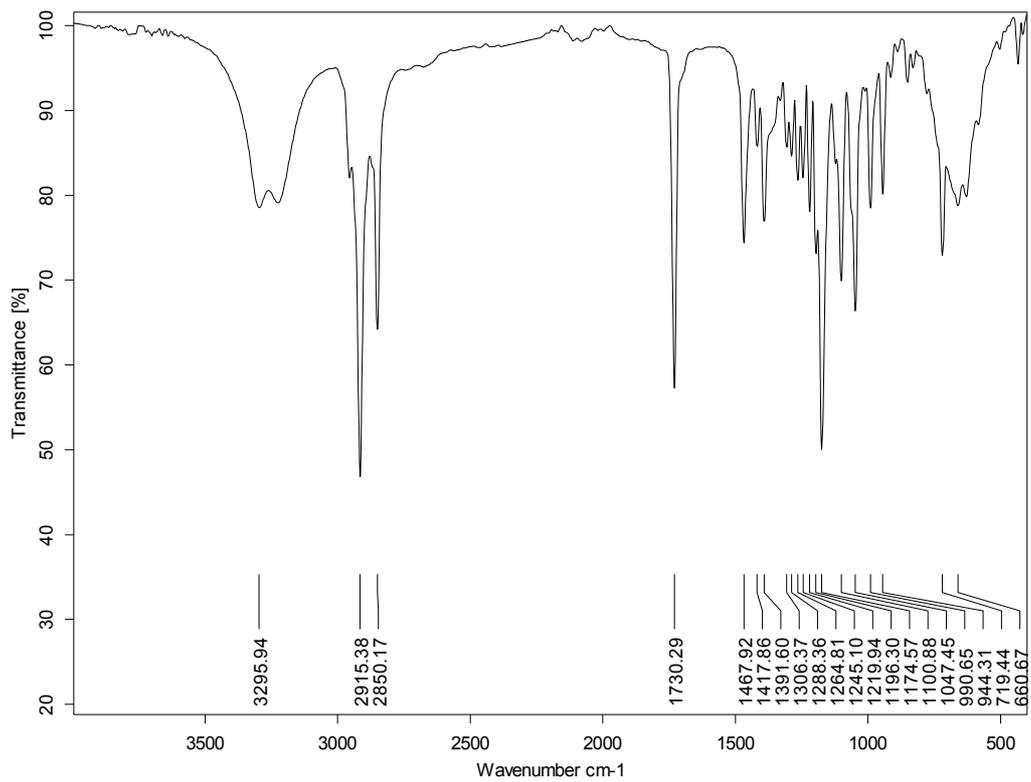


Figure 2: FTIR spectrum of the monopalmitin standard

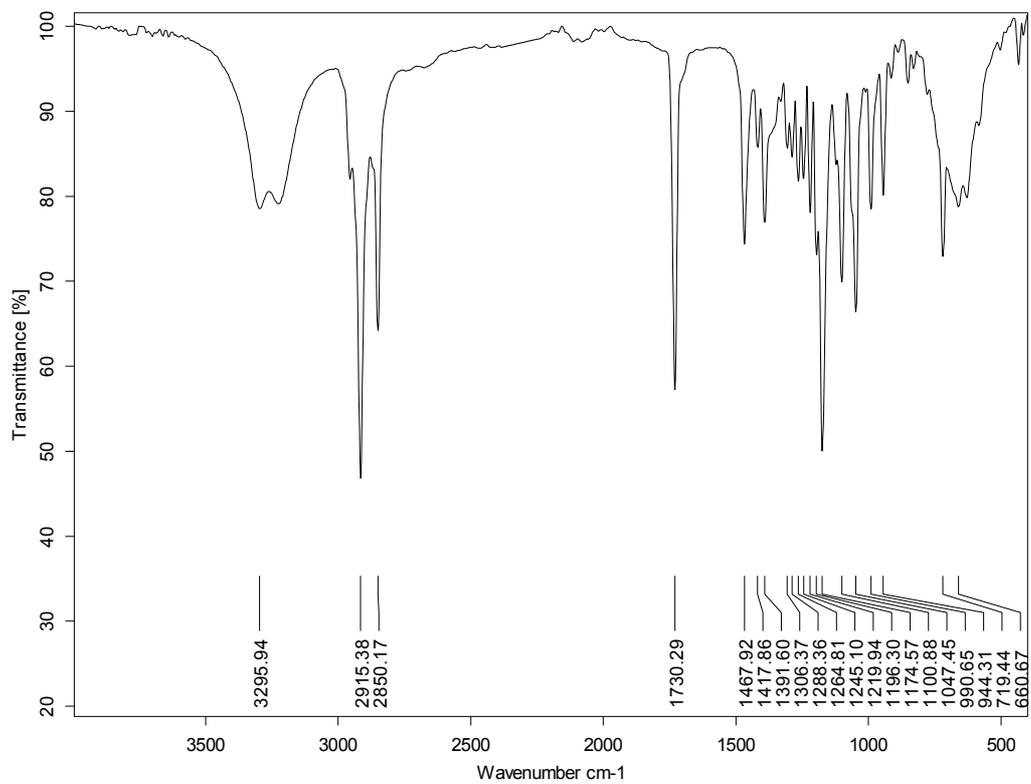


Figure 3: FTIR spectrum of F1Hz

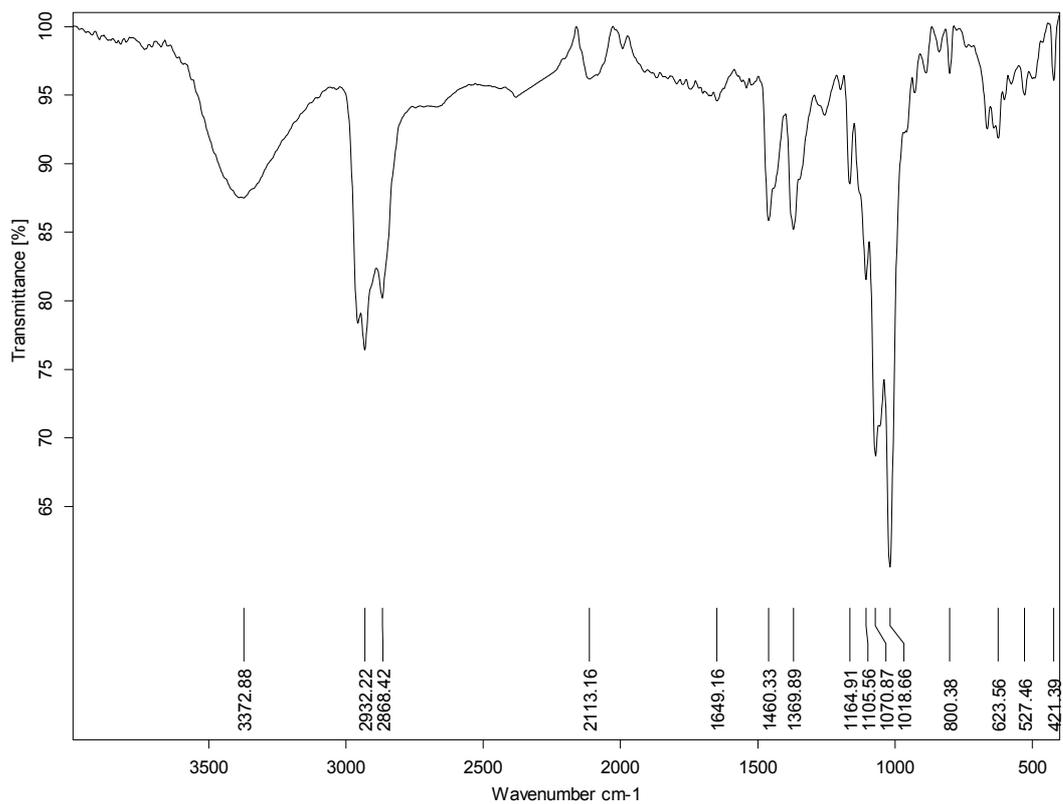


Figure 4: FTIR spectrum of the free steryl glucosides standard

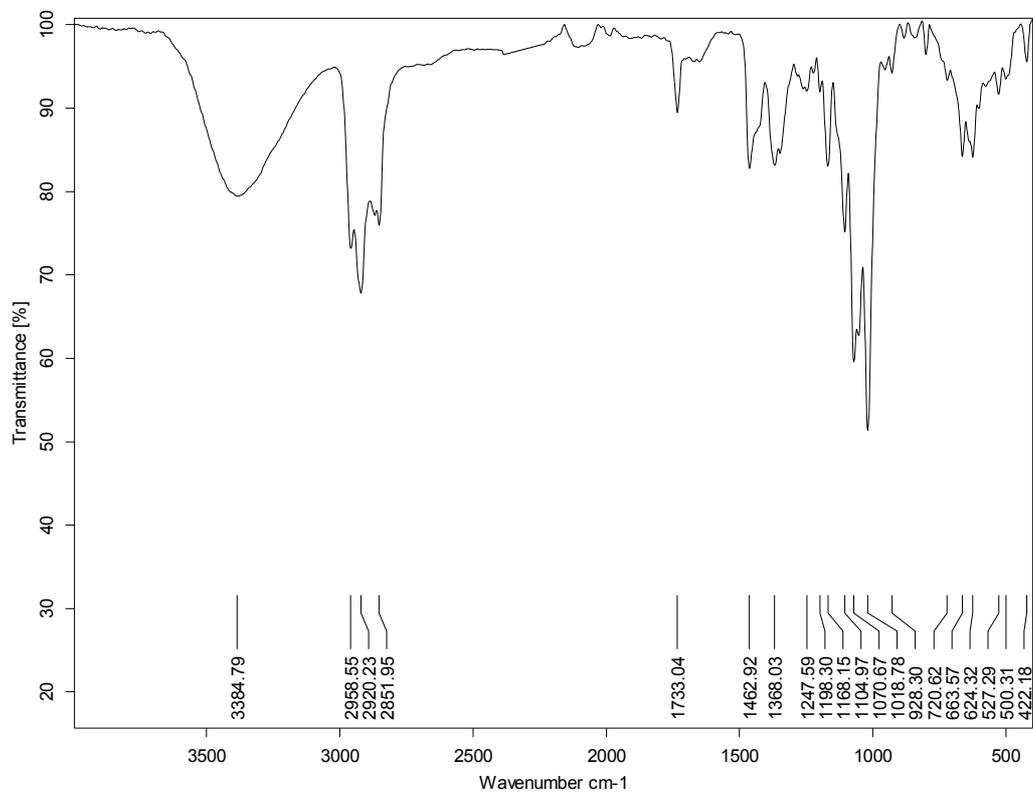


Figure 5: FTIR spectrum of F2Hz

## Acknowledgments

The authors thank Ecodiesel Colombia S.A., especially Fernando Córdoba and Gloria Suarez for providing the sample for this study. The authors also acknowledge the complete support for this research provided by COLCIENCIAS and the Colombian Petroleum Institute ICP-Ecopetrol.

## References

- Bondioli P., Cortesi N., Mariani C., 2008, Identification and quantification of steryl glucosides in biodiesel, *Eur. J. Lipid Sci. Technol.* 110, 120-126.
- Farahani M., Pagé D.J.Y.S., Turingia M.P., 2011, Sedimentation in biodiesel and ultra low sulfur diesel fuel blends, *Fuel*. 90, 951-957.
- Janssen R., Rutz D.D., 2011, Sustainability of biofuels in Latin America: Risks and opportunities, *Energy Policy*. 39, 5717-5725.
- Lacoste F., Dejean F., Griffon H., Rouquette C., 2009, Quantification of free and esterified sterylglucosides in vegetable oils and biodiesel, *Eur. J. Lipid Sci. Technol.* 111, 822-828.
- Lin H., Haagenson D.M., Wiesenborn D.P., Pryor S.W., 2011, Effect of trace contaminants on cold soak filterability of canola biodiesel, *Fuel*. 90, 1771-1777.
- Moreau R., Scott K., Hass M., 2008, The identification and quantification of steryl glucosides in precipitates from commercial biodiesel. *JAOCS*. 85, 761-770.
- Plata V., Kafarov V., Moreno N., 2010, Optimization of third generation biofuels production: Biodiesel from microalgae oil by homogeneous transesterification, *Chemical Engineering Transactions*, 21, 1201-1206.
- Plata V., Kafarov V., Castillo E., 2012, Improving the low-temperature properties and filterability of biodiesel, *Chemical Engineering Transactions*, 29, 1243-1248.
- Prada F., Ayala-Diaz I., Delgado W., Ruiz-Romero R., Romero H.M., 2011, Effect of fruit ripening on content and chemical composition of oil from three oil palm cultivars (*elaeis guineensis jacq.*) grown in Colombia, *J. Agric. Food Chem.* 59, 10136-10142.
- Tang H., Salley O.S., Simon K.Y., 2008a, Fuel properties and precipitate formation at low temperature in soy-, cottonseed-, and poultry fat-based biodiesel blends, *Fuel*. 87, 3006-3017.
- Tang H., De Guzman R.C., Salley O.S., Simon K.Y., 2008b, Formation of insolubles in palm oil-, yellow grease-, and soybean oil-based biodiesel blends after cold soaking at 4°C, *JAOCS*, 85, 1173-1182.
- Van Hoed V., Zyaykina N., De Greyt W., Maes J., Verhe R., Demeestere K., 2008, Identification and Occurrence of SterylGlucosides in Palm and Soy Biodiesel, *JAOCS*. 85, 701-709.