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Soybean Biodiesel and Metrology

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1. Introduction

Biodiesel is a renewable fuel defined as a monoalkyl ester derived from vegetable oils, animal fats or microbial oils (algae, bacteria and fungi). The conversion of the fats or oils from these raw materials into biodiesel is possible through enzymatic or chemical reactions, which the most widely employed and studied is the transesterification reaction, involving alcohol and a catalyst. Such process converts triacylglycerols into esters of fatty acids molecules, which present physical-chemical properties and cetane number similar to diesel (Krawczyk, 1996; Ma & Hanna, 1999; Li *et al.*, 2008; ASTM D6751, 2008; Moser, 2009; Knothe *et al.*, 2005; Knothe & Steidley, 2005).

Vegetable oils were first tried for combustion in engines since the early creation of Diesel engines, in the end of 19th century. At that age, the higher cost and lower availability of these oils compared to the just developed petroleum derivatives, associated to the higher homogeneity and efficiency gain up to 35% utilizing diesel, led to the complete abandonment of vegetable oils for combustion in engines. However, in the last century, the supply stability of petroleum by some countries has changed, causing drastic petroleum price raise. Thus, worldwide discussions concerning petroleum dependence were retaken, and since the second half of 90's utilization of fuels derived from renewable sources, including biodiesel, has increased in Brazil, Europe, USA and Asia (Costa *et al.*, 2003). In Brazil, social factors, such as new job opportunities, also stimulated biodiesel production.

The direct use of vegetable oils as fuel in compression ignition engines could be considered, but they are problematic due to their high viscosity (about 11-17 times greater than diesel fuel) and low volatility. These oil types do not burn completely and form carbon deposits in the fuel injectors of diesel engines. The viscosity of vegetable oils can be better improved with transesterification reaction, a process which seems to insure very good outcomes in terms of lowering viscosity and enhancing other physicochemical properties. Transesterification is a chemical reaction which proceeds under heat and involves triacylglycerols and an alcohol of lower molecular weights (typically methanol, ethanol, isopropanol or butanol) using homogeneous or heterogeneous substances as catalyst, which typically is an acid or a base, to yield biodiesel and glycerol (Ferella *et al.*, 2010), as presented in Figure 1.

Almost all biodiesel is produced from virgin vegetable oils using the base-catalyzed technique as it is the most economical process for treating virgin vegetable oils, requiring

only low temperatures and pressures and producing over 98% conversion yield (provided the starting oil is low in moisture and free fatty acids). However, biodiesel produced from other sources or by other methods may require acid catalysis which is much slower (Ataya *et al.*, 2007).

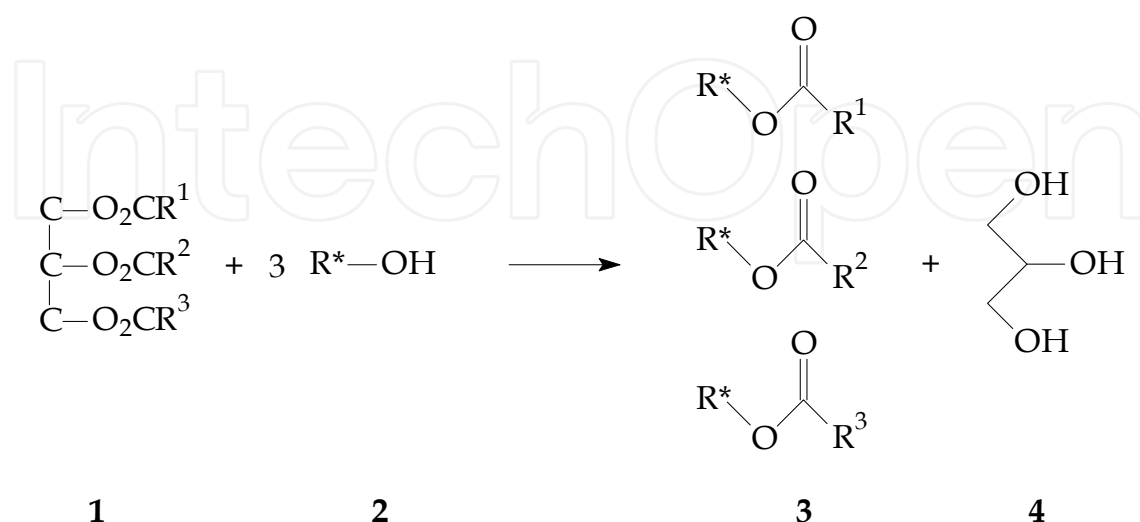


Fig. 1. A general representation of transesterification reaction between a triacylglycerols (1) and an alcohol (2) to give alkyl esters of fatty acids (3) and glycerol (4).

The purification of biodiesel is a crucial step for production of a high-quality product, and choosing of the appropriate techniques is important for this biofuel to become economically viable. Biodiesel and glycerol are typically mutually soluble, but a notable difference in density between biodiesel (880 kg/m³) and glycerol (1050 kg/m³, or more) phases is a property that allows the employment of simple separation techniques such as gravitational settling or centrifugation. Washing can be also applied to remove free glycerol, soap, excess alcohol, and residual catalyst. But in this case, drying of alkyl ester is needed to achieve the stringent limits of biodiesel specification on the amount of water (Atadashi *et al.*, 2011).

Biodiesel presents physical-chemical properties and cetane number similar to diesel, but this biofuel has several advantages over the fossil fuel (petrodiesel). Biodiesel is biodegradable, its sources are renewable, it respects the carbon cycle, and it presents lower toxicity, essentially no sulfurous and no aromatic compounds. The substitution of conventional diesel by biodiesel would reduce sulfur emissions by 20%, carbonic anhydride by 9.8%, non-burned hydrocarbonates by 14.2%, particulate material by 26.8%, and nitrogen oxide by 4.6%, thus reducing most regulated exhaust emissions. Biodiesel presents superior lubricity, higher flash point and positive energy balance (Albuquerque, 2006).

Biodiesel has been utilized blended to petrodiesel for internal combustion engines. Most countries utilize a system known as "factor B" to indicate the volumetric concentration of biodiesel in the blends. So, B100 indicates that a sample is pure biodiesel, while B20 or B5, for example, indicates that the blend has 20% (v/v) or 5% (v/v), respectively, of biodiesel. Some authors report that mixtures containing up to 20% biodiesel can generally be employed in diesel engines without modifications, but most of the authors and the vehicle produces do not recommend employing mixtures containing more than 5% biodiesel (Biodieselbr.com, 2011; Fueleconomy.gov, 2011; Biodiesel.org, 2009). Nowadays, biodiesel sold in Brazil and Europe is a B5 fuel (Biodieselbr.com, 2011; Biopowerlondon.co.uk, 2011).

However, a primary disadvantage of biodiesel is inferior oxidative and storage stability versus petrodiesel, lower volumetric energy content, reduced low temperature operability, susceptibility to hydrolysis and microbial degradation, as well as higher nitrogen oxide emissions (Albuquerque, 2006; Moser, 2009; Knothe *et al.*, 2005; Knothe & Steidley, 2005). Also, the esters which biodiesel is composed can attach to water attributing to this biofuel the hygroscopic property. Some water content also comes from the extraction and transesterification processes. The presence of water in biodiesel reduces the calorific value and enhances engine corrosion. Moreover, water promotes the growth of microorganisms and increases the probability that oxidation products are formed during long-term storage. These oxidation products can cause disturbances in the injection system and in the engine itself (Schlink & Faas, 2009).

The most significant vegetable oils produced worldwide during 2009 were palm (45.13 MMT), soybean (37.69 MMT), rapeseed/canola (21.93 MMT), and sunflower (11.45 MMT) oils (United States Department of Agriculture, 2010). Generally, the most abundant oils or fats in a region are most commonly used as feedstocks for biodiesel production. Thus, for production of biodiesel, rapeseed/canola and sunflower oils are principally used in Europe, palm oil predominates in tropical countries, and soybean oil and animal fats are most commonly used in the USA and Brazil (Moser, 2009; Knothe *et al.*, 2005).

As globalization increases, there is a need for harmonization of technical parameters regarding several products and services (especially commodities) provided around the world. In order to achieve greater transparency, reliability and suitability among these products and services, International Standards have been developed and must be followed by those countries and companies that take part in this worldwide trade. For biodiesel it is not different. Biodiesel must be certified as compliant with accepted fuel standards before combustion in diesel engines. In Europe, specifications for this biofuel are described by the European Committee for Standardization (CEN) through standard EN 14214:2008 (EN 14214:2008, 2009); in the United States the specifications must be according to ASTM D6751-08a (ASTM D6751, 2008); and in Brazil, fuels and biofuels are regulated by National Agency of Petroleum, Gas and Biofuels (ANP), through Resolution ANP no. 7 from March 19, 2008 (ANP Resolution n°7, 2008).

However, international standards are not conclusive, and many times they also are not suitable or accurate for many parameters of many products and services. Several parameters have to be exhaustively studied for the convergence of some rules. In the case of biodiesel, standards that have been applied for this biofuel were originally developed for diesel analysis and adapted for biodiesel. Just a few standards have been developed specifically for biodiesel up this time. Standards have to be constantly attending to the modernization of products, markets and methodologies. In some cases, technical methods employed to analyze some kind of product is simply adapted to analyze another similar one. An example for Biodiesel concerns the water determination method. The water content in biodiesel is ruled by EN 14214:2008 (EN 14214:2008, 2009), ASTM D6751-08a (ASTM D6751, 2008) and in Brazil by Resolution ANP no. 7 (ANP Resolution N°7, 2008). All of them settle the maximum water content as 0.05% (w/w). These standards require Karl Fischer titration for water determination, as described in ISO 12937:2000 (ISO 12937:2000, 2000). Otherwise, this ISO standard was created considering petrodiesel analyses and specifications, but now it has been adapted for water content determination in biodiesel. These adaptations of methods cause errors or at least low accuracy in analyses. Furthermore, many standards, like this one, do not describe a method setting exactly the parameters to be employed by the apparatus.

Determining such low water content in non-aqueous substances with high accuracy is not an easy task and just a few works have been published regarding moisture in biodiesel. Assessments for high accuracy determination of water in biodiesel have been performed by the Laboratory of Organic Analyses from INMETRO (Brazilian Institute of Metrology), which optimized some parameters for commercial soybean biodiesel utilizing Coulometric Karl Fischer Titration coupled to Auto-sampler Oven (Vicentim *et al.*, 2010). Experiments ongoing by this group are still verifying the necessity of further optimizations for biodiesel samples produced from another sources (data unpublished yet).

Complete discussions regarding the need for International Standards, their applications, the mechanisms for biodiesel obtainment, its chemical and physicochemical properties, and considerations about the importance of metrology and its influence on biodiesel quality will be presented in the next sessions of this chapter.

2. Biodiesel synthesis

All over the world there are many research groups searching for fuels from renewable sources due to the imminent serious depletion of fossil resources and also due to an increasing societal ecological environmental awareness. Many types of alternative energy sources have been studied, as solar, wind, water, nuclear (through the cleavage of radioisotopes) and plant biomass. However, nowadays, the only ready-to-use technologies for automotive renewable energy supply, and that has presented excellent results, are the production and utilization of the so-called biofuels, like the bioethanol from sugar cane, corn starch, sugar beet and the biodiesel, especially that one produced from oily crops.

Biodiesel can be defined as mono alkyl esters of fatty acids derived from animal fat and vegetable oils (researches are ongoing for utilization of microbial oils), and obtained mainly through the transesterification reaction. In a general way, this reaction involves triacylglycerols (which are esters) reacting with a small chain aliphatic alcohol, generally methanol, ethanol, isopropanol or butanol, producing a new ester and an alcohol, as shown in Figure 1 (Pinto et al, 2005).

Biodiesel can be derived from the following processes: pyrolysis, cracking, alcoholysis, esterification and transesterification of fats and oils which is the most commonly used process.

Processes like pyrolysis and cracking produce many side products, the reactions are not very selective and the processes require many steps, like removing ash and solid products for example. Pyrolysis, strictly defined, is the conversion of one substance into another by means of heat or by heat with the aid of a catalyst. It involves heating in the absence of air or oxygen and cleavage of chemical bonds to yield small molecules. Pyrolytic chemistry is difficult to characterize because of the variety of reaction paths and the variety of reaction products. The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids and methyl esters of fatty acids. The first pyrolysis of vegetable oil was conducted in an attempt to synthesize petroleum from vegetable oil. Since World War I, many investigators have studied the pyrolysis of vegetable oils to obtain products suitable for fuel. Catalysts have been used in many studies, largely metallic salts, to obtain paraffines and olefins similar to those present in petroleum sources. Soybean oil was thermally decomposed and distilled in air and nitrogen sparged with a standard ASTM distillation apparatus. The total identified hydrocarbons obtained from the distillation of soybean and high oleic safflower oils were 73-77 and 80-88% respectively. The main components were alkanes and alkenes, which

accounted for approximately 60% of the total weight. Carboxylic acids accounted for another 9.6-16.1% (Fangrui & Milford, 1999).

Esterification is a process that consists in two main steps. In the first one the oil is saponified with sodium hydroxide followed by acidification, washing and drying, obtaining a mix of fatty acids. In the final steps the fatty acids are esterified with a small chain alcohol, like methanol, ethanol or isopropyl alcohol.

2.1 Transesterification using catalysts

In a general way transesterification reaction occur catalyzed by an acid (Gerpen, 2005), alkali (Rinaldi et al, 2007), enzyme (Mendes et al, 2011 & Watanabe et al, 2002) or employing heterogeneous catalysis (Mell et al, 2011). The main heterogeneous catalysts are zeolites (Suppes et al, 2004), clays (Jaimasith et al, 2007), ion-exchange resins (Honda et al, 2007) and oxides.

The most used way of catalysis is employing an alkali. The reaction mechanism under alkaline condition occurs in two steps: In the first step sodium hydroxide reacts with methanol, in an acid-base reaction producing a strong base, sodium methoxide and water. In the second step sodium methoxide reacts as a nucleophile and attacks the three carbonyl carbons from the triacylglycerol. A very unstable tetrahedral intermediate is obtained. As a result, the cracking of the triacylglycerol occurs, obtaining three methyl esters (biodiesel) and glycerol.

The most employed transesterifying agent is methanol. Other alcohols may also be used in the preparation of biodiesel, such as ethanol, propanol, isopropanol, and butanol. Ethanol is of particular interest primarily because it is less expensive than methanol in some regions of the world, and biodiesel prepared from bioethanol is completely bio-based. Butanol may also be obtained from biological materials, thus yielding completely bio-based biodiesel as well. Methanol, propanol, and isopropanol are normally produced from petrochemical materials such as methane obtained from natural gas in the case of methanol. Some conditions utilized in these reactions are described below:

Methanolysis: The classic reaction conditions for the methanolysis of vegetable oils or animal fats are 6:1 molar ratio of methanol to oil, 0.5 wt.% alkali catalyst (with respect to TAG), 600 rpm, 60°C reaction temperature, and 1 h reaction time to produce FAME and glycerol.

Ethanolysis: The classic conditions for ethanolysis of vegetable oils or animal fats are 6:1 molar ratio of ethanol to oil, 0.5 wt.% catalyst (with respect to TAG), 600 rpm, 75°C reaction temperature, and 1 h reaction time to produce fatty acid ethyl esters (FAEE) and glycerol.

Butanolysis: The classic conditions for butanolysis of vegetable oils or animal fats are 6:1 molar ratio of butanol to oil, 0.5 wt.% catalyst (with respect to TAG), 600 rpm, 114°C reaction temperature, and 1 h reaction time to produce fatty acid butyl esters and glycerol. Butanol is completely miscible with vegetable oils and animal fats because it is significantly less polar than methanol and ethanol. Consequently, transesterification reactions employing butanol are monophasic throughout. The monophasic nature of butanolysis reactions also complicates purification of the resultant butyl esters (Moser, 2009).

2.1.1 Homogeneous catalysts

Conventional processes include the use of homogeneous alkaline catalysts – NaOH, KOH, NaOMe and KOMe – under mild temperatures (60–80 °C) and atmospheric pressure. There are two main factors that affect the cost of traditional biodiesel production: the cost

of raw materials and the cost of processing (multiple steps), though the commercialization of resultant glycerol can share the production costs with biodiesel, improving the overall process profitability. In order to reduce the costs associated with feedstock, waste cooking oils, animal fats or non-edible oils could be used. However, the use of homogeneous alkaline catalysts in the transesterification of such fats and oils involves several troubles due to the presence of large amounts of free fatty acids (FFAs). Of course, alkaline catalysts can be used to process these raw materials, but a large consumption of catalyst as well as methanol is compulsory to achieve biodiesel of standard specifications. Thus, FFA concentration in the oil inlet stream is usually controlled below 0.5% (w/w), avoiding the formation of high soap concentrations as a consequence of the reaction of FFAs with the basic catalyst. The soap causes processing problems downstream in the product separation because of emulsion formation. Usually, this problem is overcome through a previous esterification step where FFAs are firstly esterified to FAMES using a homogeneous acid catalyst, and then, once the acid homogeneous catalyst has been removed, transesterification of triacylglycerols is performed as usual by means of an alkaline catalyst. Likewise, homogeneous acid catalysts (H_2SO_4 , HCl , BF_3 , H_3PO_4) have been proposed to promote simultaneous esterification of FFAs and transesterification of triacylglycerols in a single catalytic step, thus avoiding the pre-conditioning step when using low cost feedstock with high FFA content. However, these catalysts are less active for transesterification than alkaline catalysts and therefore higher pressure and temperature, methanol to oil molar ratio and catalyst concentration are required to yield adequate transesterification reaction rates. Hence, despite its insensitivity to free fatty acids in the feedstock, acid-catalyzed transesterification has been largely ignored mainly due to its relatively slower reaction rate. (Melero et al, 2009).

2.1.2 Heterogeneous catalysts

The use of heterogeneous catalysts (Wang et al, 2007 and Leclercq et al, 2001) has as main advantage the reaction work-up, the post reaction treatment, the purification steps and the separation steps. These catalysts can be easily removed from the reaction medium and even can be reused. Another interesting factor is based in the fact that these catalysts avoid the formation of undesirable side products, like the saponification products (Botts et al, 2001; Thomasevic & Marinovic, 2003). The biggest difficulty at this type of reaction is the diffusion between the systems oil/catalyst/solvent (Gryglewics, 1999). For the soybean biodiesel production, the catalysts that have been commonly employed are tin, zinc and aluminum, as Al_2O_3 , ZnO and $(Al_2O_3)_8(SnO_2)$, for example (Mello et al, 2011). Other processes have used an heterogeneous catalyst of a spinel mixed oxide of two (non noble) metals, which eliminate several neutralization and washing steps needed for process using heterogeneous catalysts (Helwani et. al, 2009).

2.1.3 Alkaline catalysts

Alkaline catalysis (Zhou et al, 2003) is a procedure that generally uses sodium and potassium alkoxides, and some times sodium and potassium hydroxides or carbonates. Among these three groups, alkoxides have the advantage of performing reactions at mild temperatures, they provide high yields of esters derived from fatty acids and they are not corrosives like the acid catalysts. On the other hand, these catalysts are hygroscopic, more expensive and usually result in side products, such as the saponification ones.

2.1.4 Acid catalysts

Sulfuric and hydrochloric acid compounds are the main catalysts. This catalysis (Mohamad and Ali, 2002) has the advantage of avoiding the formation of side products and obtaining high yield formation of alkyl esters. However, reactions in acid media are highly corrosive and the work up is more difficult, seen it needs a special treatment to neutralize the reaction medium

2.1.5 Enzymes catalysts

A fourth class of catalysts employed for biodiesel production is enzyme (Fukuda et al, 2001). The enzymes allow the use of mild temperature reactions, between 20 and 60 °C, excess of alcohol is dispensed, the reactions can be performed with or without a solvent and the catalyst can be reused several times (Shimada et al, 2002). Other great advantages are the easy work-up, dispensing neutralization and deodorization of the reaction medium (Bielecki et al, 2009). The disadvantages are related to the fact that enzymes are very specific, expensive and very sensible to alcohols, causing their deactivation (Manduzzi et al, 2008). The literature (Modi et al, 2007) shows that this problem can be solved by the use of small amounts of water (Kaieda et al, 2001 and Ban at al, 1999). Another research group showed that the use of organic solvents (Narasisham et al, 2008) can activate the enzymes, in special the use of dioxane and petroleum ether (Dennis et al, 2008), for example. Watanabe and his research group has developed a methodology to produce biodiesel from soybean degummed oil by the use of the lipase (an enzyme specific for hydrolysis of lipids, like triacylglycerides) from *Candida antarctica* in a free solvent system (Watanabe et al, 2002). Another procedure was performed by Liu et al. (2005) studying the acyl group migration with immobilized lipozyme TL catalyzing the production of biodiesel from soybean oil (Noureddini et al, 2005).

2.2 New process for biodiesel obtainment

2.2.1 Microwave and Ultrasound

Many researches seek for the improvement of catalysts in biodiesel production. Reactions employing ultrasound (Santos et al, 2009) and microwave (Leadbeater & Stencel, 2006) techniques represent a great advance. Ultrasound (Chand et al, 2010) and microwaves (Barnard et al, 2007) as auxiliary techniques facilitate the interaction between methoxide ions and reagents, increasing the process efficiency, obtaining higher yields in a shorter reaction time. Reaction employing these techniques can be performed at mild temperatures due to a higher kinetic energy in the reaction medium, facilitating also the miscibility among the reactants (Fukuda, 2001).

2.2.2 Transesterification using supercritical fluids

This is a non catalytic method to produce biodiesel, which has the several advantages. One of them concerns the shorter reaction time than the traditional catalyzed transesterification. This is possible because the initial reaction lag time is overcome due to the reaction is proceeded in a single homogeneous phase since the supercritical methanol is fully miscible with the vegetable oils. Moreover, the reaction rate is very high and the subsequent purification is much simpler than that of the conventional process. The supercritical route is also characterized by high yield because of simultaneous transesterification of triacylglycerols and esterification of fatty acids. This process is environmentally friendly

seen that waste water containing alkali or acid catalysts is not produced. The disadvantages of this process regard the high costs, the necessity of a high pressure system (200-400 bar), high temperatures (350-400°C) and high methanol/oil rates (Balat, M.H., 2008; Melero et al, 2009).

2.2.3 Hydrotreating

Hydrotreating is a process that produces biodiesel through a hydrotreatment of triacylglycerols. The hydrocarbons are produced by two reaction pathways: hydrodeoxygenation (HDO) and hydrodecarboxylation (HDC). n-Alkanes originating from HDO have the same carbon number as the original fatty acid chain, i.e., even carbon number, typically 16 or 18. Water and propane are the main reaction by-products of this route (Snare et al, 2007).

2.3 Biodiesel purification process

Separation and purification of biodiesel is a critical task. Normally, the crude biodiesel produced by homogeneous catalysis can be separated from glycerol by simple gravitational settling or centrifugation, due to their notable difference in phase density (biodiesel 880 kg/m³ and glycerol 1050 kg/m³ or more). Washing ester phase with water or an acid mineral or base solution to remove base/acid catalyst residues, for example, can be also applied to remove free glycerol, soap, excess alcohol, and residual catalyst. Finally the biodiesel is dried after neutralization. For methanol recycle vacuum distillation can be used prior to glycerin purification. When the biodiesel is obtained by a heterogeneous catalysis, this one is removed by a filtration process.

However, these conventional technologies and other ones like decantation, washing with ether and the use of absorbents have proven to be inefficient, time and energy consumptive, and less cost effective. On the other hand, the involvement of membrane reactor and separative membrane shows great promise for the separation and purification of biodiesel. Membrane technology needs to be explored and exploited to overcome the difficulties usually encountered in the separation and purification of biodiesel (Zhang et al, 2003; Atadashi et al., 2011).

3. Biodiesel properties and their influence on engine performance

3.1 The fatty acid composition of feedstocks and the influence on the properties of biodiesel fuel

The most common feedstocks for biodiesel production are commodities such as vegetable oils derived from soybean, palm and sunflower seed. These materials possess fatty acid profiles consisting primarily of five fatty acids with carbon chains containing 16 to 18 carbon atoms (C16 to C18) namely palmitic acid (hexadecanoic-C16: 0), stearic acid (octadecanoic, C18: 0), oleic acid (9 (Z)-octadecenoic - C18: 1), linoleic (9 (Z), 12 (Z)-octadecadienoic acid - C18: 2), linolenic (9 (Z), 12 (Z), 15 (Z) , octadecatrienoic acid- C18: 3). The proportions of different fatty acids in feedstocks influence the properties of biodiesel. Some of the most relevant properties to be considered for a biodiesel candidate to be used as a substitute for diesel fuel (or blended with the same) are cetane number, viscosity, cold flow properties and oxidative stability. Lubricity is another important parameter for a fuel but it is independent on the fatty acid composition.

Two major problems to be overcome in biodiesel are the poor properties at low temperatures and low oxidative stability. In most cases these two problems occur with the same sample. They result from physical and chemical properties of fatty esters, the major components of biodiesel and minor constituents that arise during the transesterification reaction or are from raw materials.

The profile of methyl esters found in greater proportion in soybean is about 11% C16:0, 4% C18:0, 21-24% C18:1, 49-53% C18:2, 7-8% C18:3 which provides cetane number in the range of 48-52, kinematic viscosity at 40 °C equal to 4.10 to 4.15 mm²s⁻¹ and cloud point approximately equal to 0 °C (Knothe et al., 2005, Mittelbach and Remschmidt, 2004). Rapessed (canola) methyl esters have a fatty acid profile approximately 4% C16:0, 2% C18:0, 58-62% C18:1, 21-24% C18:2, 10-11% C18:3 and present cetane number in the range of 51-55, kinematic viscosity at 40 °C around 4,5 mm²s⁻¹ and cloud point of approximately -3 °C (Knothe et al., 2005, Mittelbach and Remschmidt, 2004). Thus the difference in fatty acid profile, more specifically concerning C18:1 and C18:2 contents, which had their values almost reversed in the case presented, causes a noticeable change in fuel properties.

Many researches have focused on resolving or at least reducing problems related to low oxidative stability and cold flow properties of biodiesel. Some trials in this way involves the addition of additives and changes in the composition of fatty esters, that can be reached varying either the reactive alcohol or the oil fatty acid profile. Changing the fatty acid profile can be achieved by physical methods, genetic modification of feedstock or use of alternative feedstocks with different fatty acid profiles.

Important features regarding the use of neat biodiesel or its blends with diesel fuel include reduced emissions, with the exception of nitrogen oxides, compared to petrodiesel (petroleum-derived diesel fuel), biodegradability, absence of sulfur, inherent lubricity, positive energy balance, higher flash point, compatibility with existing infrastructure for distribution of fuel, to be renewable and a domestic source. The American ASTM D6751-08a, the European EN 14214:2008 and the Brazilian ANP n^o7 standards deal with the technical specifications for biodiesel to be used in internal combustion cycle diesel engine taking into account the advantage of utilizing the existing infrastructure for distribution of diesel ensuring fuel quality for the final consumer. Table 1 shows the specifications recommended by American, European and Brazilian standards aiming biodiesel utilization as fuel.

3.2 The influence of cetane number on combustion and atmospheric emissions

The cetane number (CN) is a dimensionless parameter related to the ignition delay time after fuel injection into the combustion chamber of a diesel engine. A higher cetane number results in a shorter ignition delay time and vice versa. A cetane scale was established, being hexadecane commonly used as reference compound, with CN = 100, and 2,2,4,4,6,8,8-heptamethylnonane, a highly branched compound with poor ignition quality in a diesel engine, with CN =15.

The cetane scale explains why the triacylglycerols, such as those found in vegetable oils, animal fats and their derivatives, are suitable alternatives to diesel fuel. The reason is the long chain, linear and unbranched fatty acids, chemically similar to those in n-alkanes of conventional diesel fuels with good quality.

The cetane number of fatty esters increases with the increase of saturation and carbon chain. Thus, the CN of methyl palmitate and methyl stearate (C16:0 and C18:0) is greater than 80 (Knothe et al., 2003), the CN of methyl oleate (C18:1) is in the range of 55-58, the methyl

linolenate is (C18: 2) around 40 and the methyl linolenate is (C18: 3) around 25. Esters derived from branched alcohols such as isopropanol have CN values comparable to methyl esters or other ester with alkylic chain (Knothe et al., 2003, Zhang & Gerpen, 1996) linear, although the cost of production once isopropyl alcohol is more expensive than methanol and ethanol costs.

In general biodiesel does not require additives to improve cetane number, because its cetane number generally reaches the minimum values established in the international technical specifications. An exception may be the methyl esters of soybean that did not reach the minimum of 51 set by EN 14214:2008 (EN 14214:2008, 2009) but usually reach the minimum set of 47 recommended in ASTM D6751 (ASTM D6751-08a, 2008), as shown in Table 1.

Cetane number may influence both the quality of combustion and vehicle emissions. Several international agencies like the EPA (Environmental Protection Agency - USA) and the CONAMA (National Environment Council - Brazil) set limits and goals for reducing pollutants automotive emissions. In diesel cycle engines, the main pollutants are hydrocarbons, carbon monoxide, nitrogen oxides (NO_x) and particulate matter. Reducing these emissions requires improving the combustion process, the treatment of exhaust gases from existing engines and technical fuels specifications. A low cetane number leads to difficulties in cold starting, increases emissions and noise level of combustion. If the cetane number is high may occur an increase in particulate emissions but NO_x emissions decrease. Samples of biodiesel with low level of triacylglycerols, especially those with polyunsaturated fatty acids of C18:3, should show low levels of NO_x emissions. Linear correlation was obtained between the level of unsaturation of biodiesel indicated by iodine number, the density of biodiesel and NO_x emissions (McCormick et al., 2001). Thus little amounts of unsaturated fatty acids may reduce the density and the NO_x emissions. An important property of biodiesel is its ability to reduce total particulate emissions of the engine and also carbon monoxide and hydrocarbons contents of exhaust gases. However biodiesel causes an increase in NO_x emissions. Increasing CN to a certain level (around 60) implies in the reduction of NO_x emissions (Landommatos et al., 1996).

An experiment was conducted with the OM 611 diesel engine light load of Damler Benz with ultra low sulfur content diesel (ULSD), conventional diesel and B20 blend of pure methylic soybean biodiesel and ULSD. The results obtained with the B20 blend showed no differences in NO_x content compared to the two reference diesel fuels. Reductions of particulate matter by 32% and 14%, respectively, compared to conventional diesel fuel and ULSD were observed with B20 blend (Sirman, et al., 2000).

The causes for the increase of NO_x associated with biodiesel for fuel injection systems are related to a small displacement in the range of fuel injection which is caused by differences in mechanical properties of biodiesel compared to conventional diesel (Tat & van Gerpen, 2003; Monyem et al., 2001). Due to the higher modulus of compressibility (or sound speed) of biodiesel, there is a faster transfer of the pressure wave of the injection pump to the injector needle resulting in anticipation of lifting the needle and the production of a small advance in the injection interval. It was observed that samples of B100 derived from soybeans produces an increase of one degree in the injection interval, which was accompanied by a four degree at the start of combustion (Sybist & Boehman, 2003). Strategies that can be used to reduce NO_x emissions to a level equivalent to that of conventional diesel involve increase of cetane number by use of additives.

Specification	Standards					
	ASTM D6751-08a		EN 14214:2008		ANP n ^o 7	
	Test method	Limit	Test method	Limit	Test method	Limit
Cetane number	ASTM D613; D6890	47 minimum	EN ISO 5165	51 minimum	ASTM D613; D6890 EN ISO 5165	Report
Kinematic viscosity	ASTM D445	1.9-6.0 mm ² s ⁻¹	EN ISO 3104	3.5-5.0 mm ² s ⁻¹	ASTM D445; EN ISO 3104; ABNT NBR10441	3.0-6.0 mm ² s ⁻¹
Oxidative stability	EN 14112	3h minimum	EN 14112	6h minimum	EN 14112	6h minimum
Cloud Point	ASTM D2500	Report	-	-	-	-
Cold filter plugging point	-	-	EN 116	Depending on time of year and location	ASTM 6371; EN 116; ABNT NBR14747	19 °C
Cold soak/Filterability	Annex to D6751	Report	-	-	EN 12662	24 mg/Kg (max.)

ASTM - American Society for Testing and Materials; ISO - International Standards Organization; ANP - National Agency of Oil, Gas and Biofuels ; NBR - Brazilian Standard; ABNT - Brazilian Association of Technical Standards

Table 1. Specifications of biodiesel standards that affect the properties of alkyl esters as fuel in diesel cycle engines ^(a).

It was observed that B20 blends of soy diesel respond well to conventional peroxide di-t-butyl, a cetane improver, when tested on DDC Series 60 engines of 1991 (McCormick , et al., 2001). The biodiesel NO_x was reduced by 6.2% without the contribution of 9.1% in reducing emissions of particulate matter to be compromised and B20 blend produced no noticeable increase in NO_x of this engine. The peroxide, di-t-butyl nitrate and 2-ethylhexyl were tested in a similar engine (Sharp, 1994) and the reduced levels of NO_x in exhaust emissions were confirmed. Notice the economy of this procedure if necessary high levels of additives.

3.3 The importance of viscosity in the use of biodiesel as fuel

Viscosity is one of the properties that most affect the use of biodiesel as a fuel since the atomization process, the initial stage of combustion in a diesel engine, is significantly affected by the viscosity of the fuel. The viscosity of the transesterified oils, ie, biodiesel is less than their vegetable oil sources, which explains the failure to use pure vegetable oils as alternative fuels to diesel. The high viscosity of untransesterified oils leads to operational problems in diesel engine for example increased engine deposits. Viscosity in the form of Kinematic viscosity is specified in quality standards of biodiesel, which exhibit a range with

minimum and maximum values for this parameter. Although there are standardized methods for determining the kinematic viscosity as shown in Table 1, several studies have been conducted to predict the viscosity of biodiesel from its composition of fatty ester.

Assuming a soy biodiesel made from soybean oil containing 0.1% C14: 0, 10.3% C16: 0, 4.7% C18: 0, 22.5% C18: 1, 54.1% of C18: 2 and 8.3% of C18: 3, Allen et al., 1999 using the equation of Grunberg & Nissan, 1949, modified, predicted the value of $3.79 \text{ mm}^2\text{s}^{-1}$ for the viscosity of methyl soybean biodiesel. The viscosity of fatty esters increases with the chain length and with increasing degree of saturation (Kern and Van Nostrand, 1949).

This rule also applies to alcohol used in the reaction, since the viscosity of ethyl esters is slightly higher than that of methyl esters. The configuration of double bonds also influences the viscosity. If there are only double bonds in cis configuration is observed remarkable reduction in viscosity, as well as esters with double bonds in the trans configuration have viscosities similar to the corresponding saturated esters (Kern & Van Nostrand, 1949).

3.4 Cold flow properties of biodiesel and its blends with diesel

3.4.1 Biodiesel and cold flow properties

The mixtures such as biodiesel do not possess defined melting points, but melting ranges. This fact reflects in the specifications used in biodiesel standards.

The cloud point (CP) is the temperature at which the first solids appear, but the fuel can still flow, although these solids can lead to fuel filter plugging (Dunn and Bagby, 1995).

The pour point, usually a few degrees below the cloud point, is the temperature at which the fuel can no longer be freely poured.

Several other methods exist for determining the low-temperature properties of biodiesel. These are the cold filter plugging point (CFPP) and low-temperature flow test (LTFT) (Dunn and Bagby, 1995). The CP and CFPP are included in biodiesel standards without severity since in ASTM D6751 for the value of CP only a report is required and the CFPP value in EN 14214 can vary with time of year and geographic location. The low-temperature properties of biodiesel are also influenced by the properties of individual components. The melting point of fatty esters generally increase with chain length (although chains with odd numbers of carbon have slightly lower melting points than the preceding even-number chain) and increasing saturation (Knothe, 2009).

Intending to provide the industry with an independently generated set of cold flow information on a variety of fuels in the market of the United States in 2009 with the new Ultra Low Sulfur Diesel Fuel (ULSD), cloud point, cold filter plugging point (CFPP) and low temperature flow test (LTFT) methods were used to assess the cold-flow properties for seven different biodiesel fuels blended with four different ULSD fuels representing the span of the market in 2009 (Heck, Thaeler, Howell and Hayes, 2009). The neat fuels were tested in addition to biodiesel blends with ratios of 2% biodiesel (B2), 5% biodiesel (B5), 11% biodiesel (B11), 20% biodiesel (B20) and 50% biodiesel (B50) for cloud point, CFPP, and LTFT. The pour point of the neat biodiesel and B50 blends were also analyzed. Three petrodiesel fuels with cloud points of $-47.5 \text{ }^\circ\text{C}$, $-16 \text{ }^\circ\text{C}$ and $-11 \text{ }^\circ\text{C}$ were used to produce petrodiesel having target cloud points of $-40 \text{ }^\circ\text{C}$, $-34.4 \text{ }^\circ\text{C}$, $-26.1 \text{ }^\circ\text{C}$, and $-12.2 \text{ }^\circ\text{C}$. Seven biodiesel (B100) samples were selected. Three of them with low cloud point, vegetable oil base, in this case soybean oil from various manufacturing processes (distilled biodiesel, non distilled biodiesel from hexane extracted oil, non distilled biodiesel from extruder-expeller oil) were collected from commercial biodiesel producers. Four additional biodiesels of mid to mid-high to high cloud

points from commercial biodiesel producers representing mixed saturation levels, blends of soybean, animal, and recycled oil based biodiesel. The cloud point values of the various B100 samples were -2,5 °C, -2,0 °C, -1,5 °C, 1,0 °C, 7,0 °C, 8,0 °C, and +12,0 °C. In some cases the impact on cold flow properties of blending biodiesel with petrodiesel appeared to be mostly linear, while in others the impact was curvilinear. In all cases except a few with low blends of biodiesel where the CFPP of the blend was slightly below that of the petrodiesel, the blended fuel values fell between the pure petrodiesel and pure biodiesel values for all three cold flow measurements.

Biodiesel blends primarily B20 of soybean biodiesel (SME) and diesel n₂ have also been used in a variety of climates including some of the coldest weather on record without cold flow problems. A study to determine the CFPP of blends containing up to 20% (SME) and Number 2 (n₂) diesel was conducted. The University of Missouri prepared the samples that were analyzed in the Cleveland Technical Center in Kansas City, United States. The characteristics of the n₂ Diesel and the SME are shown in Table 2. The results suggest that the blends with the highest content of biodiesel begin to gel first. Higher concentrations of biodiesel, eg, above 20% may not be suitable for use in cold climates without mixing large quantities of kerosene in combination with cold flow proven enhancers specific to the conventional diesel (National Biodiesel Board- 2007/2008).

Blends's components	Cloud Point (°C)	Pour Point (°C)	CFPP (°C)
n ₂ Diesel	-15,6 °C	-34,4 °C	-17,2 °C
Soy Methyl Esters (SME)	0 °C	-3,9 °C	-5,6 °C

Table 2. Characteristics of the components of the blends.

Literature data show that ethyl esters and specially isopropyl esters improve low-temperature properties of biodiesel compared to methyl esters. Isopropyl and isobutyl esters of common soybean oil exhibited crystallization temperatures 7-11 and 12-14 °C lower than the corresponding methyl esters (Lee et al., 1995). The data suggest that the fuel blend that begins to gel first contains the highest concentration of biodiesel. Higher concentrations of biodiesel, eg, above 20% may not be suitable for use in cold climates without mixing large quantities of kerosene in combination with proven enhancers of cold-flow properties specific to the conventional diesel.

3.4.2 Diesel fuel background information relevant to biodiesel

The cold weather operability of diesel fuel is defined as the lowest temperature which a vehicle will operate without loss of power due to waxing of the fuel delivery system. Diesel fuels composition and cold flow properties vary greatly across the United States. Cold flow characteristics of diesel fuels are influenced by the source of the crude oil they are made from, how they are refined and if they are blended to improve performance during cold weather. The cold temperature properties of diesel fuel vary across the country depending on the time of year the fuel is produced and the climate. Generally, diesel fuels used in cold climates have better cold flow characteristics than diesel fuels used in warmer regions. Both of these statements have a direct impact on the operability of biodiesel blends in cold weather.

The refining process separates the crude oil into mixtures of its constituents, based primarily on their volatility. Diesel fuels are on the heavy end of a barrel of crude oil. This gives diesel

fuel its high BTU content and power, but also causes problems with diesel vehicle operation in cold weather when this conventional diesel fuel can gel. This is not an issue for gasoline vehicles. A tremendous amount of effort has been spent over the years to understand how to deal with the cold flow properties—or the low temperature operability—of existing petroleum based diesel fuel. The low temperature operability of diesel fuel is commonly characterized by the cloud point, and the cold filter plugging point (CFPP) or the low temperature filterability test (LTFT). In general, Number 2 diesel fuel will develop low temperature problems sooner than will Number 1 diesel fuel. Number 1 diesel fuel is sometimes referred as kerosene. The gelling of diesel fuel in cold climates is a commonly known phenomenon and diesel fuel suppliers, as well as customers and diesel engine designers, have learned over time to manage the cold flow problems associated with Number 2 diesel fuel in the winter time. The leading options to handle cold weather with diesel fuel are: -Blending with kerosene;-Utilization of an additive that enhances cold flow properties;-Utilization of fuel tank, fuel filter or fuel line heaters;-Storage of the vehicles in or near a building when not in use. In most diesel engine systems today, excess diesel fuel is brought to the engine and warm fuel that has come close to the engine is recycled back to the fuel tank. This assists in keeping the fuel from gelling in cold weather. This is, in part, why diesel engines are kept running overnight at truck stops in cold climates (Bickell, 2008; Krishna & Butcher, 2008; Joshi & Pegg, 2007).

3.4.3 The impact of minor components of the vegetable oils in the cold flow properties of biodiesel

The presence of sterol glucosides (SGs), wax, monoglycerides, saturated fatty acids and polymers in both B100 and blends with petrodiesel can limit the application of these fuels due to problems with precipitation. In the last years researches have shown that biodiesel precipitations can arise even if specifications of this biofuel are met. Special attention has been dedicated to the SGs, components commonly found in vegetables and in oils derived from soybean, rapeseed and palm that will be processed to produce biodiesel. Usually the concentration of SGs in vegetable oils is not significant since they are mostly found as sterol glucosides acylated (ASGs). The ASGs have average solubility in vegetable oils, but after transesterification they are broken down chemically by removing the side chain containing the fatty acid and they are converted partially to SGs. This class of compounds is not soluble in biodiesel and its crystallization is extremely slow and depends on temperature, other impurities (as crystallization nuclei) and surface effects. However, even a brand new biodiesel, meeting all the specifications, presents precipitation of SGs after a few days of storage/transport.

The spontaneous clogging of the filters in the production unit or in the supply chain has been observed in Minnesota, United States with a B2 blend of soybean methyl esters and diesel. Several other places in the world have also observed this occurrence with B5 blend. The precipitates do not contain only SGs but also ASGs and other substances. In some cases sources containing higher concentrations of ASGs could be responsible for the deposits. The concentration of SGs and ASGs in vegetable oils depends on the feedstock and on the process used by industry to obtain them. The literature suggests that the highest concentrations of SGs and ASGs will be found in soybean and palm oil. Rapeseed oil usually has low concentrations of these compounds. In order to minimize the effect of SGs and ASGs on the FAME-Biodiesel (Fatty Acid Methyl Esters -biodiesel) and their blends with

petrodiesel, special treatments of the esters in an oil refinery, or the use of adsorbents could be possible solutions for a post-processing of FAME (Haupt et al., 2009).

3.5 The effect of the impurities in the quality of biodiesel

Biodiesel is composed by alkyl esters (generally methyl esters) that can be analyzed as tool for controlling the transesterification yield. Low concentration of triacylglycerols is an indicative that transesterification is almost complete. Seen that this kind of reaction is reversible, excess of alcohol must be added to ensure that transesterification will prone to esters production. If great amount of alcohol remains in the biodiesel its flash point decreases and problems with storage and transport can occur. Glycerine is a by-product of transesterification that must be recovered in order to avoid solid in diesel engines. Standard methods that must be employed to determine triacylglycerols, alcohol (methanol), total ester and glycerine are presented in Figure 2.

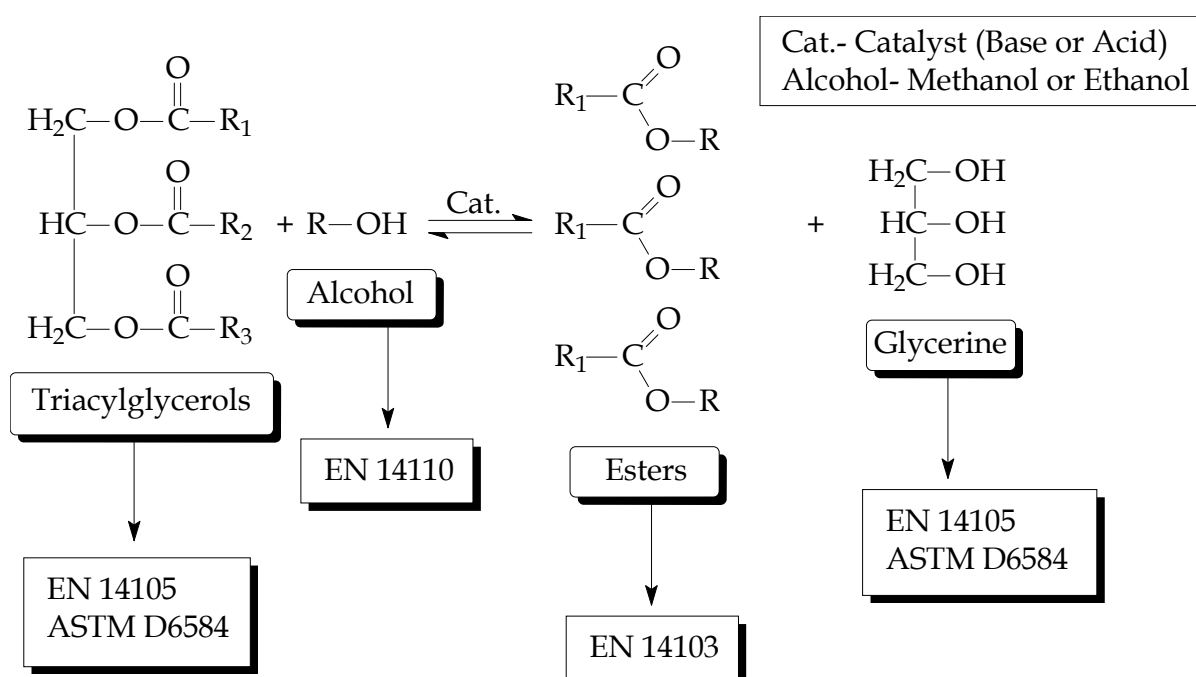


Fig. 2. Methods to quantify some organic impurities present in biodiesel.

Transesterification reaction proceeds in three main steps, shown in Figure 3. Firstly the triacylglycerols are transformed into diacylglycerols and then, these ones are converted into monoacylglycerols, which in turn reacts with alcohol yielding glycerine and an ester. Glycerine can be present in biodiesel in a free form or combined with glycerides. The total glycerine is the sum of these 2 glycerine forms. Maximum limits of methanol, glycerides, free and total glycerine contents in biodiesel, as standard methods for determination of these parameters are shown in Table 3.

All the methods described in Table 3, for determination of the concentration of the organic compounds in biodiesel, employ gas chromatography. So, a typical soybean biodiesel chromatogram, acquired in accordance with EN 14105 standard, is presented in Figure 4. This chromatogram shows the peak of free glycerin (1) and of the internal standards (butanetriol (2) and tricaprine (5)) utilized to quantify free glycerine and mono, di and triglycerides, respectively. It is also observed the regions where the methyl esters (3),

monoglycerides (4), diglycerides (6) and triacylglycerols (7) are eluted. This standard method was developed for rapeseed methyl esters determination, but they have been applied successfully for the same determination in soybean and sunflower derivate. In Brazil, Resolution ANP nº7 demands the method validation when EN 14105 is employed to analyze biodiesel samples derived from feedstocks other than rapessed, or when biodiesel was produced from by ethylic route.

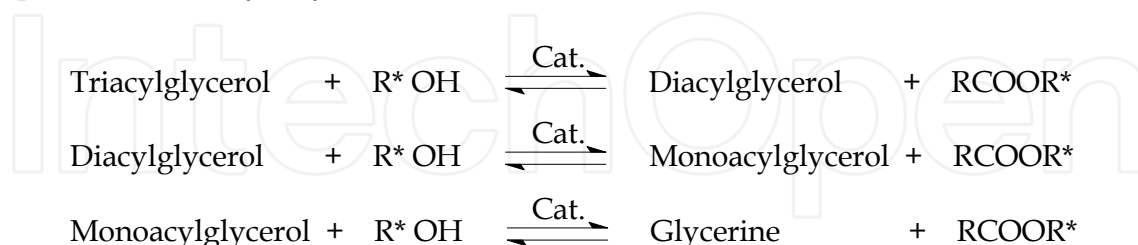


Fig. 3. Steps of the transesterification reaction.

Specification	Standards					
	ASTM D6751-08a		EN 14214:2008		ANP n º7	
	Test method	Limit (g/100g)	Test method	Limit (g/100g)	Test method	Limit (g/100g)
Methanol	EN 14110	0,20 max.	EN 14110	0,20 max.	EN 14110	0,20 max.
Free glycerine	ASTM D6584	0,02 max.	EN 14105	0,02 max.	ASTM D6584 EN 14105	0,02 max.
Monoglycerides	-	-	EN 14105	0,80 max.	ASTM D6584 EN 14105	0,80 max.
Diglycerides	-	-	EN 14105	0,20 max.	ASTM D6584 EN 14105	0,20 max.
Triglycerides	-	-	EN 14105	0,20 max.	ASTM D6584 EN 14105	0,20 max.
Total glycerine	ASTM D6584	0,24	EN 14105	0,25 max.	ASTM D6584 EN 14105	0,25 max.

ASTM - American Society for Testing and Materials; ISO - International Standards Organization; ANP - National Agency of Oil, Gas and Biofuels

Table 3. Methods and limits of the impurities present in biodiesel.

3.6 Oxidative stability

The oxidation of fatty acid chain is a complex process proceeded by a variety of mechanisms. Oxidation of biodiesel is due to the unsaturation in fatty acid chain and presence of double bonds in the molecule which offers high level of reactivity with O₂, especially, when it is placed in contact with air/water. The primary oxidation products of double bonds are unstable allylic hydroperoxides which are unstable and easily form a variety of secondary oxidation products. This includes the rearrangement of product of similar molecular weights to give short chain aldehydes, acids compounds and high molecular weight materials.

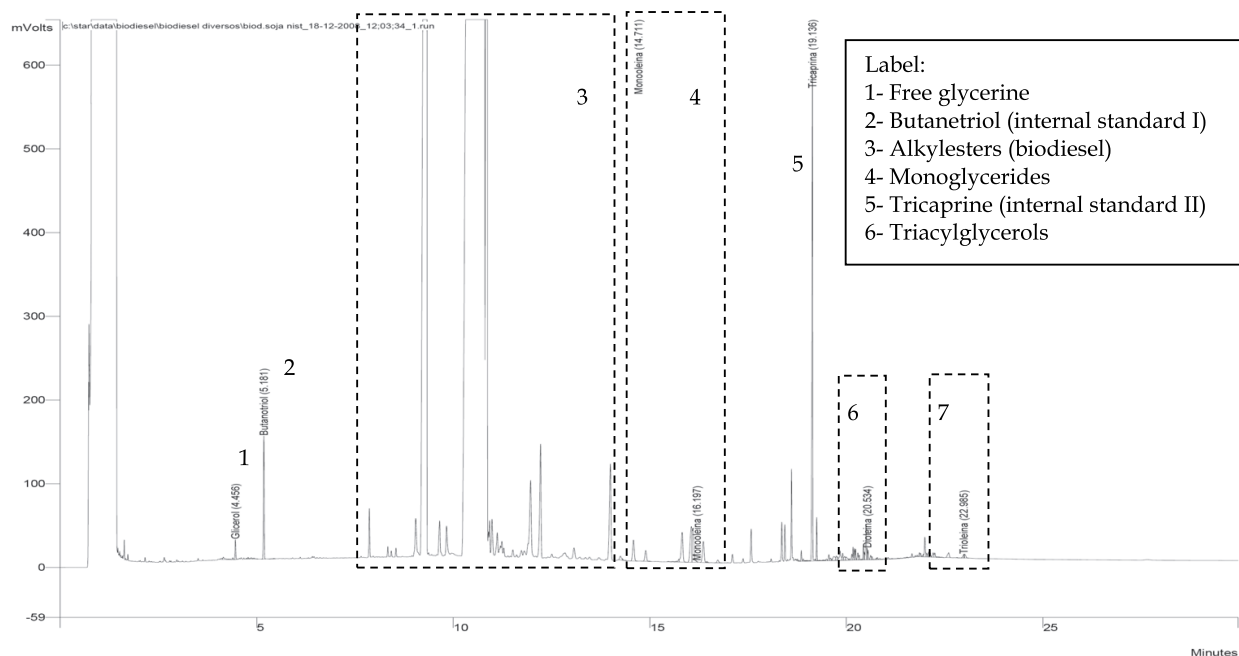


Fig. 4. Soybean biodiesel chromatogram obtained in accordance with EN 14105 standard (Source: Organic Analysis Laboratory- INMETRO - 2008).

Peroxidation occurs by a set of reactions categorized as initiation, propagation, and termination, as shows Figure 5. The reaction mechanism involved in the first step is the removal of hydrogen from a carbon atom to produce a carbon free radical. If diatomic oxygen is present, the subsequent reaction to form a peroxy radical becomes extremely fast, not allowing significant alternatives for the carbon-based free radical. The peroxy free radical is not reactive compared to carbon free radical, but is sufficiently reactive to quickly abstract hydrogen from a carbon to form another carbon radical and a hydroperoxide (ROOH). The new carbon free radical can then react with diatomic oxygen to continue the propagation cycle. This chain reaction terminates when two free radicals react with each other to yield stable products.

Fatty oils that contain more poly-unsaturation are more prone to oxidation. Literature reveals the relative rate of oxidation for the methyl esters of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids to be 1:12:25 (Siddharth & Sharma, 2010).

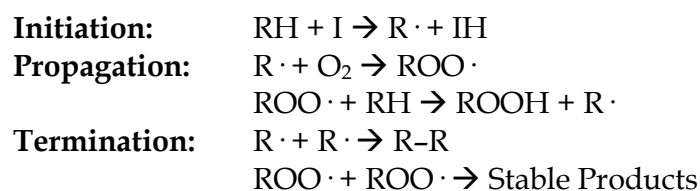


Fig. 5. Mechanism of peroxidation of fatty acids

The biodiesel oxidative stability study is a very important parameter to measure the product quality, mainly about its feedstock. This parameter is a measure of time required to reach the point where the oxidation increases sharply. This methodology is useful to determinate the final biodiesel stability under several oxidative conditions. Useful appropriate oxidative automatic techniques are Petrooxy, differential scanning calorimetry (DSC), Pressure

Differential Scanning Calorimetry (PDSC) (Dufaure et al, 1999) and mainly Rancimat technique. At the Rancimat technique, oxidative stability is based on the electrolytic conductivity increase (Hadorn & Zurcher, 1974.). The biodiesel is prematurely aged by the thermal decomposition. The formed products by the decomposition are blown by an air flow (10L/ 110 °C) into a measuring cell that contains bi-distilled, ionized water. The induction time is determined by the conductivity measure and this is totally automatic. Rancimat is the most used technique to determine finalized biodiesel stability, under oxidative accelerated conditions, according to standard EN14112.

At the PetroOxy Technique, the sample is inducted to oxidation through an intense oxygen flow, manipulating by this way the stability conditions through a specific apparatus. The analysis time is recorded as the required time to the sample absorbs 10% of oxygen pressure. The differential scanning calorimetry (DSC) monitors the difference in energy provided/released between the sample (reagent system) and the reference system (inert) as a function of temperature when both the system are subjected to a controlled temperature program. Changes in temperature sample are caused by rearrangements of induced phase changes, dehydration reaction, dissociation or decomposition reactions, oxidation or reduction reaction, gelatinization and other chemical reactions.

The Pressure Differential Scanning Calorimetry (PDSC) is a thermo analytical technique that measures the oxidative stability using a differential heat flow between sample and reference thermocouple under variations of temperatures and pressure. This technique differs from the Rancimat for being a fast method and presents a more variable - the pressure, allowing to work at low temperatures and using a small amount of sample (Candeia, 2009).

3.6.1 Antioxidants used in biodiesel

Most of biodiesel has a lower value of oxidative stability than recommended by current legislation (Ji-Yeon, 2008 & Ferrari, 2009) (Table 4), the soybean derivative has also the same inconvenient. This characteristic is due to the rich composition in mono and polyunsaturated fatty acids from the soybean oil.

Source of Biodiesel	Oxidative Stability (h)
Sunflower	1,17
Jatropha	3,23
Soybean	3,87
Palm	11,00

Table 4. Oxidative stability of biodiesel samples produced from different sources.

Compounds containing allylic and bis-allylic have greater reaction fragility with oxygen due to the formation of stable resonance structure, as shows Figure 6.

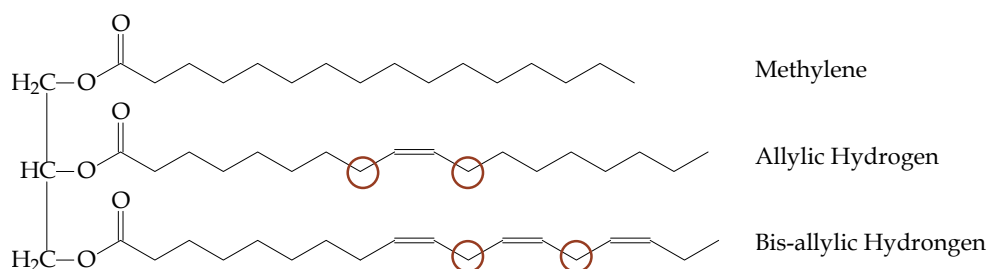


Fig. 6. Methylenic ,Allylic and bis allylic hydrogens at triacylglycerol (Asadukas et al, 2007).

The allylic hydrogen reactivity is 40 times greater than the methylene hydrogen and the bis-allylic is 100 times more reactive than the methylene hydrogen (Knothe, 2007).

The main characteristic for a substance be considered a good antioxidant is its capacity to react with oxygen faster than the biodiesel components, mainly the unsaturated compounds. Moreover, the radicals generated in this reaction have to be stable enough and less reactive with the initial biodiesel components or even with the generated products from the biodiesel reaction.

Denisov & Khudyakov (1987) divide the antioxidants class in four groups:

Group 1 - Inhibitors that terminate chains through reactions with peroxy radicals, including phenols, aromatic amines, diamines, and aminophenols;

Group 2 - Inhibitors that terminate chains through reactions with alkyl radicals, including stable radicals, quinones, quinone imines, methylenequinones, nitro compounds, and condensed aromatic hydrocarbons (these inhibitors are effective when dissolved oxygen concentration is low);

Group 3 - Agents that decompose peroxides without generating free radicals, including sulfides, disulfides, phosphites, metal thiophosphates, and carbamates;

Group 4 - Complexing agents that deactivate heavy metals are capable of catalyzing hydroperoxide decomposition to free radicals, thereby promoting oxidation, including diamines, amino acids, hydroxy acids and other bifunctional compounds.

Compounds from the group 3 contain sulfur that turn difficult its use as biodiesel for environmental reasons. Compounds from the group two are effective only for oxygen low concentrations.

Actually there are two substances classes are very useful for this purpose: phenols and aromatics amines. These compounds are cheap and very useful at oil and polymers industry and are the most useful at biodiesel industry.

Figure 7 shows the oxidative stability of biodiesel containing different types of phenol antioxidants. Its stability can be up to 5 times higher when *tert*-Butylhydroquinone (TBHQ) is added, Karavalis, 2011.

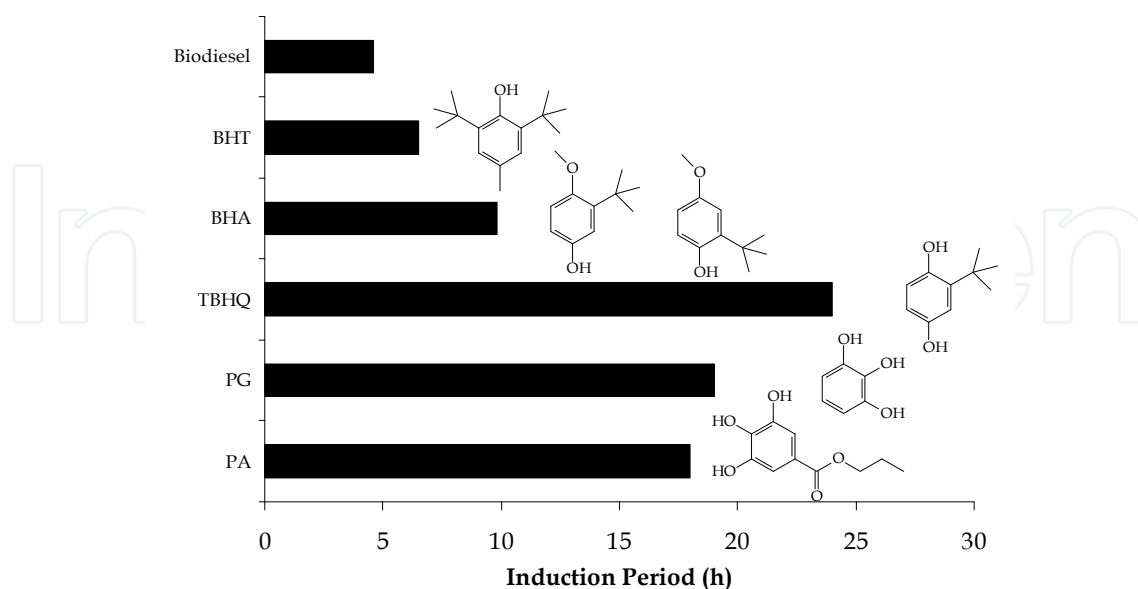


Fig. 7. PA= Propylgallate (3,4,5-trihydroxybenzoate) ; PG= Pyrogallol (benzene-1,2,3-triol) ; BHA= mixture of the isomers 2 and 3-*tert*-butyl-4-hydroxyanisol; BHT= di-*tert*-butyl-metil-phenol (Butylated hydroxytoluene) ; TBHQ= *tert*-Butylhydroquinone.

4. The importance of metrology for biodiesel quality

4.1 Efforts for harmonization of biodiesel standards

In 2006, the Government of Brazil, the European Commission (representing the European Union) and the Government of the United States of America, during trilateral discussions, affirmed their belief that the current market for biofuels is viable. The market will continue to grow within these regions and the international trade in biofuels would increase significantly by the end of this decade (Tripartite Task Force, 2007). However, a potential barrier to global trade in biofuels concerns the differences among the standards describing and ruling their composition and properties.

To overcome these potential barriers, a conference was organized by the European Commission and the European Committee for Standardization (CEN), with the active participation of the U.S. National Institute of Standards and Technology (NIST) and the Brazil's National Institute of Metrology, Standardization, and Industrial Quality (INMETRO). This meeting, held in Brussels in February, 2007, convened a broad range of private-sector biofuels experts and government representatives from the EU, US and Brazil. The participants confirmed that differing standards for biofuels were a potential handicap to the free circulation of biofuels among the three regions.

To support the global trade of biofuels, representatives of Brazil, the EU and the U.S. agreed to promote, whenever possible, the compatibility of biofuels-related standards in their respective regions. Such compatibility would not only facilitate the increasing use of biofuels in each of the regional markets, but also would support both exporters and importers of biofuels by helping to avoid adverse trade implications in a global market. Subsequently, the International Biofuels Forum (IBF) - a governmental initiative among Brazil, China, the European Commission, India, South Africa, and the United States - was launched in March, 2007 to promote the sustained use and production of biofuels around the globe. The IBF also concluded that trade will play an increasing role in providing adequate supplies of biofuels to the markets where the energy demand for transport fuel is rising at an accelerated rate.

In June, 2007, a NIST and INMETRO-sponsored Biofuels Symposium in Washington, DC, convened representatives from Brazil, the EU and the U.S. to build on the work begun in Brussels. These representatives agreed to review existing documentary standards for biofuels and identify areas where greater compatibility could be achieved in the short, medium and long term. According to the tripartite agreement, the standards to be considered were those produced by Brazilian Association for Technical Standards (ABNT), Brazilian Petroleum, Gas and Biofuels Agency (ANP), European Committee for Standardization (CEN) and American Society for Testing and Materials (ASTM International) and in effect before the end of 2007. It was further agreed that only standards pertaining to the biofuels being currently traded - biodiesel and bioethanol - would be addressed; this was further limited to pure biofuels and not to ready-made blends.

Comprised of representatives from the private and public sectors, the Biodiesel Tripartite Task Force and the Bioethanol Tripartite Task Force each started their technical work in July. The immediate task was to classify the various specifications into three categories:

- Category A: specifications that are already similar;
- Category B: specifications with significant differences between parameters and methods, but which might be aligned by work on documentary standards and measurement standards; and

- Category C: specifications with fundamental differences, perhaps due to emissions or environmental regulations within one or more regions, which are not deemed bridgeable in the foreseeable future.

There were commonalities with the approach and methodology used by both of the Task Forces. Each of the two groups assembled and translated existing standards from ABNT, ASTM International and CEN, and the units for specifications were converted to a common basis. Each Task Force first compared the standards as they presently exist. Since it was noted that many parameters were different, the Task force members entered into discussions and negotiations and were able to make specific recommendations to address these differences. They further agreed that these recommendations should be forwarded to standards bodies for consideration and possible implementation. Here, we will only present all biodiesel discussions to compatibility biodiesel standards. Summary results from each group are listed below in Table 5.

4.2 General considerations for biodiesel standards

The current standards established to govern the quality of biodiesel on the market are based on a variety of factors which vary from region to region, including characteristics of the existing diesel fuel standards, the predominance of the types of diesel engines most common in the region, and the emissions regulations governing those engines. Europe, for example, has a much larger diesel passenger car fleet, while United States and Brazilian markets are mainly comprised of heavier duty diesel engines. It is therefore not surprising that there are some significant differences among the three sets of standards.

Category A Similar	Category B Significant Differences	Category C Fundamental Differences
Sulfated Ash	Total glycerol content	Sulfur content
Alkali and alkaline earth metal content	Phosphorus content	Cold climate operability
Free glycerol content	Carbon residue	Cetane number
Copper strip corrosion	Ester content	Oxidation stability
Methanol and ethanol content	Distillation temperature	Mono, di, and tri-acylglycerides
Acid number	Flash point	Density
	Total Contamination	Kinematic viscosity
	Water content and sediment	Iodine number
		Linolenic acid content
		Polyunsaturated methyl ester

Table 5. Classification of the Various Biodiesel Specifications.

Other sources of regional differences in biodiesel standards arise from the following factors. The biodiesel standards in Brazil and the U.S. are applicable for both fatty acid methyl esters (FAME) and fatty acid ethyl esters (FAEE), whereas the current European biodiesel standard is only applicable for fatty acid methyl esters (FAME). Also, the standards for biodiesel in Brazil and the U.S. are used to describe a product that represents a blending component in conventional hydrocarbon based diesel fuel, while the European biodiesel standard describes a product that can be used either as a stand-alone diesel fuel or as a blending component in conventional hydrocarbon based diesel fuel.

It should also be noted that some specifications for biodiesel are feedstock neutral and some have been formulated around the locally available feedstocks. The diversity in these technical specifications is primarily related to the origin of the feedstock and the characteristics of the local markets. Though this currently translates into some significant divergence in specifications and properties of the derived fuels – which could be perceived as an impediment to trade – in most cases it is possible to meet the various regional specifications by blending the various types of biodiesel to the desired quality and specifications.

The Task Force members classify the various specifications according with the limits for each parameter. They have collaboratively assembled a definitive and widely vetted list of Brazilian, EU and US standard specifications that are similar. In addition, they have identified a list of specifications that have significant, but alignable differences. Perhaps even more importantly, some indirect benefits have been derived. There is widespread agreement amongst the participating experts that the discussions and commitment to cross-border cooperation have been a major accomplishment that will support the increase in global trade of biofuels. The experts now have a better understanding of reasons why regional differences exist, and a new atmosphere of collegiality has been created – not only between countries but also between the private and public sector representatives. These positive outcomes foster a working environment that will support ongoing movement towards enhanced compatibility among the biofuels standards.

After the discussions, it was concluded that:

- standardization bodies of the tripartite agreement (i.e., ABNT, ANP, CEN and ASTM International) as a basis for ongoing discussions and cooperation that will promote alignment and mitigate divergence among evolving standards and specifications.
- other members of the International Biofuels Forum as a basis for ongoing discussions on more closely aligning their respective specifications and prioritizing future efforts for maximum impact.
- request the standardization bodies of the Tripartite Agreement to consider adapting existing national standards wherever appropriate. Furthermore the standardization bodies should attempt where possible, when developing and updating their standards on biodiesel from now on to consider the opportunity to align with the other standards in question;
- support efforts to initiate an analysis of the categorized specifications to study trade implications and appropriate next steps for harmonization;
- support the development of internationally-accepted reference methods and certified reference materials for improving the accuracy of measurement results that underpin assessment of product quality, and help facilitate trade.

4.3 Development of Internationally-accepted reference methods and certified reference materials

Beyond the difference among the standards, an unacceptable barrier to trade, are the measurement disagreements between countries. To overcome such problems it is necessary to have an international infrastructure within which it is possible to make comparable measurements (Wielgoz & Kaarls, 2009).

This is true for all areas of measurements including chemical ones. Such a system requires measurement standards that have long-term stability and are internationally recognized. The International System of Units (SI) represents such a system, and by the use of traceable measurements provides an international infrastructure for comparable measurements. This system is demonstrated in Fig. 8.

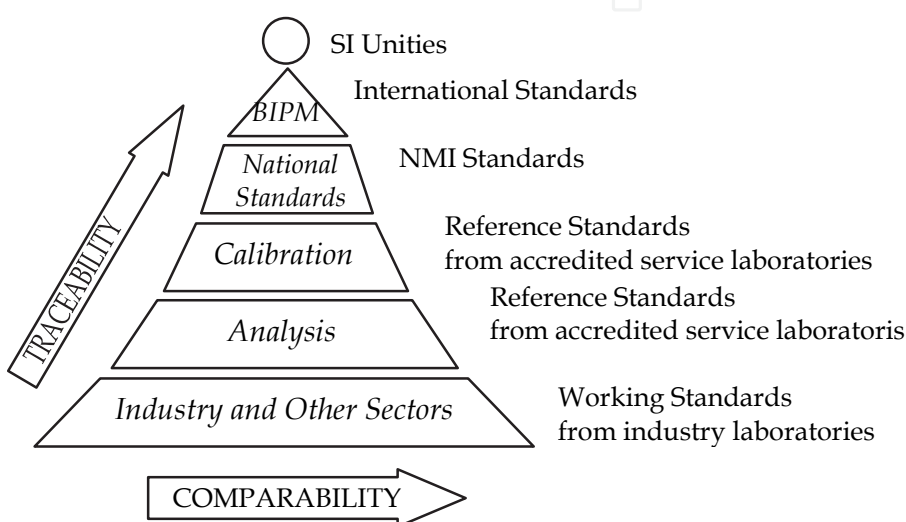


Fig. 8. Traceability scheme.

The International System of Units (SI) is at the top of the system (Dube, 2001). Its units are realized by standards. A measurement is a process, in the course of which the measurand is compared to a standard. For practical measurements, usually a working standard not a primary standard is used. To state the uncertainty of the measurement result, the uncertainty of the value assigned to the working standard must be known. It results from the uncertainty of the comparison measurement of the working standard with the reference standard. The uncertainty of the value assigned to the reference standard results from the uncertainty of the comparison measurement of the reference standard with the primary standard. This chain of comparison measurements is exactly what the definition of the term “traceability” means. If the traceability of a measurement result is guaranteed, its uncertainty can be stated. From this considerations it follows that metrology can provide the tools, necessary to get reliable measurement results.

Metrology is the science of measurement, embracing both experimental and theoretical determinations at any level of uncertainty in any field of science and technology. Within a robust metrological system the values of measurement standards and measurement results are linked *via* comparisons or calibrations which take into account the measurement uncertainty of the linking processes. Measurement uncertainty is the parameter associated with the results of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand.

The ISO Guide to the Expression of Uncertainty in Measurement (GUM) (BIPM et al., 2008) and the Eurachem (CITAC, 2000) guide on measurement uncertainty provide guidance on the evaluation of measurement uncertainty. The property of the result of a measurement or the value of a measurement standard whereby it can be related to stated references, usually national or international measurement standards, through an unbroken chain of comparisons all having stated uncertainties, is termed (metrological) traceability. Where these stated references are realizations of the SI units the term SI-traceable is used. Traceability is the basis of the comparability of a measurement: whether the result of a measurement can be compared to the previous one, a measurement result a year ago, or to the result of a measurement performed anywhere else in the world. Traceability is most often obtained by calibration, establishing the relation between the indication of a measuring instrument and the value of a measurement standard.

In the field of analytical chemistry the term Certified Reference Material (CRM) is more often used than measurement standard. A CRM is a reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.

Metrological traceability (Eurachem/CITAC, 2003) may also be established to a reference method, defining the measurand and fixing a number of influence parameters, the results of which are expressed in SI units, and an approach that has been documented for the field of laboratory medicine. In the field of metrology in chemistry the role of a National Metrology Institute (NMI) involves: realization, maintenance and dissemination of the units; development and application of primary measurement methods; establishment of traceability structure, guaranteeing the equivalence of measurement standards using programs which facilitate traceable measurements to be achieved, including the provision of certified reference materials, both as pure materials and calibration solutions as well as matrix reference materials for method validation or calibration.

For producers of CRMs, there are three ISO Guides that assist the set-up of a facility to produce and certify RMs and to ensure that the quality of thus-produced CRMs meet the requirements of the end-users (ISO, 2006). ISO Guide 34 (ISO, 2009) outlines the requirements to be met by a CRM producer to demonstrate competence, whereas the Guide 35 provides assistance on how to meet these requirements. At a fairly generic level, this Guide provides models for homogeneity testing, stability testing, and the characterization of the candidate CRM.

ISO Guide 31 (ISO, 2000) describes the format and contents of certificates for CRMs. In some ways, this Guide can be seen as an application of the *Guide to the Expression of Uncertainty in Measurement* (GUM) with respect to the peculiarities of the production of CRMs. Where possible, the Guide 35 makes reference to the GUM, as the latter describes in detail how to evaluate measurement uncertainty of a value obtained from measurement. This Guide complements the GUM in a sense that it provides additional guidance with respect to the inclusion of the uncertainties due to the (remaining) batch inhomogeneity and instability of the CRM in the uncertainty of the property values, and the determination of these uncertainty contributions.

Thorough knowledge of the material and its properties, and of the measurement methods used during homogeneity testing, stability testing and characterization of the material, along with a thorough knowledge of the statistical methods (Eurachem/CITAC, 2000), are

needed for correct processing and interpretation of experimental data in a typical certification project. It is the combination of these required skills that makes the production and certification of RMs so complex. The greatest challenge in these projects is to combine these skills to allow a smooth implementation of RM certification.

Three categories of values can be assigned for certified reference materials producers: Certified values fulfill the highest standards for reliability. They are traceable to stated references and are accompanied by a GUM (BIPM, JCGM 100:2008) compatible expanded uncertainty statement valid for the entire shelf life of the CRM. Indicative values are not certified due to either a larger uncertainty than required for the intended use or insufficient variety of methods used in the characterization. The information is therefore unsuitable for certification at the accuracy required for certified values. Additional material information are values created during the certification exercise, which are usually the result of one method only and indicate the order of magnitude rather than an accurate value.

In summary, certified values are those values the certifying body is confident in assigning with the highest accuracy, while indicative values display higher uncertainties and/or lack a full traceability statement. This hierarchy in reliability is shown by the fact that only certified values are on the first page of the certificate. It follows that certified values are more assured than indicative values which in turn are more assured than additional material information.

The measurement method used for the homogeneity study should have very good repeatability and selectivity. The main purpose of the homogeneity assessment is, however, to detect unexpected problems, for example due to contamination during packaging (Linsinger et al., 2000, Van der Veen et al., 2000, 2001a, 2001b). To establish the homogeneity, a statistically defined number of bottles is randomly selected and analyzed for all relevant property values. For evaluation of homogeneity results, unifactorial analysis of variance ("one-way ANOVA") (Van der Veen & Pauwels, 2000) is applied. The stability of the reference materials have to be assessed for all parameters, by measuring the property values periodically during the course of the project. In this case, where samples are measured on different days, the selectivity and the reproducibility of the measurement method are of great importance. Therefore, methods for homogeneity and stability studies are not necessarily the same. This is not a problem so long as traceability of the results of the homogeneity and stability studies and characterization to a common reference are established. Such a reference may be a material that is suitable for assessing the various calibrations or results from different measurement methods. Ensuring the traceability of all measurements in a certification project is an important requirement.

For the characterization of the candidate reference material, the producer shall use and document technically valid procedures to characterize its reference materials. It shall comply with the requirements of ISO Guide 35 and ISO/IEC 17025 for testing, calibration and related activities. There are several technically valid approaches for characterizing a reference material. These include carrying out measurements using: a) a single (primary) method in a single laboratory; b) two or more independent reference methods in one or several laboratories; c) one or more methods of demonstrable accuracy, performed by a network of competent laboratories; d) an approach providing method-specific, operationally defined property values, using a network of competent laboratories.

Depending on the type of reference material, its intended use, the competence of the laboratories involved and the quality of methods employed, one approach may be chosen as appropriate. Results obtained from proficiency testing can be used only if the competence of

the laboratories involved has been checked and it has been ensured that the measurements done comply with ISO/IEC 17025. The single (primary) method approach shall be carried out only when the procedure and expertise enable it to ensure metrological traceability. More usually, a property value can be reliably assessed when its value is confirmed by several laboratories working independently and using more than one method, for each of which the accuracy has been well established.

Primary methods play an essential role in the practical realization of the base units of the SI and hence in establishing traceability to the SI throughout metrology (Milton, 2001). There are seven SI units (meter, kilogram, second, ampere, Kelvin, mole, candela). They are the essential first link in such a chain of traceability because they do not require prior knowledge of any measurement of the same quantity. Explained briefly, a primary method of measurement allows a quantity to be measured in terms of a particular SI unit without reference to a standard or measurement already expressed in that unit. It is thus, in principle, completely independent of measurements of the same quantity, but calls upon measurements expressed in other units of the SI. By their nature, primary methods are unbiased (their results are accurate) but they may not necessarily be precise. Those primary methods that are at the same time precise are the ones that are useful in practice. Put in a different way, a primary method provides the means to transform the abstract definition of an SI unit into practical measurements made in terms of that unit. This is sometimes referred to as a "realization" of that unit, but this statement can be confusing because it gives the impression that the result is in some way a concrete materialization of the unit. In some cases such a concrete materialization can be made (although it is not possible for the mol unit) but, more importantly, a primary method allows measurements to be made in terms of that unit and this is the main characteristic of a primary method. The potential primary methods available for the chemical area are very few, they are: Gravimetry, Titrimetry, Coulometry, Calorimetry (Differential Scanning Calorimetry), Isotope Dilution Mass Spectrometry (IDMS), Instrumental Neutron Activation Analysis (INAA).

The biggest challenge involving the characterization of a biodiesel CRM is the lack of primary methods for all parameters that involves the biodiesel quality assessment. Therefore, some efforts are being made in that sense. Inmetro and NIST have teamed up to develop a CRM for several parameters for biodiesel derived from soybean and animal fat. The composition of the materials had to be close to specification levels, for those parameters where international agreement on these levels exists. Characterization is carried out for those parameters where SI traceability of the measurement results is possible. NIST and Inmetro reported the results accompanied by a complete uncertainty statement calculated according to the "Guide to the expression of uncertainty in measurement" (GUM) (BIPM, JCGM 100:2008). These CRMs are available at NIST homepage.

Additional effort concerns BIOREMA project (Inmetro et al., 2008) which involved not only Inmetro and NIST, but also several European National Metrology Institutes as Laboratory of Government for Chemistry - LGC and National Physical Laboratory- NPL (England), Van Swinden Laboratory - VSL (Netherlands), Institute for Reference Material and Measurements - IRMM (Belgium). Initially, this group intended to develop a (Certified) Reference Material for biodiesel from rapeseed/canola and conducted homogeneity, stability and characterization studies in test samples to obtain certified values.

However NMI's results for several parameters were not harmonized as expected, so these test samples were not possible to be used as CRM. On the other hand, these results were not dismissed, and they can be used as reference value for intercomparisons. Thus, the

BIOREMA group decided to promote a proficiency testing within Brazilian, American and European testing laboratories. In this proficiency testing, soybean and animal fat biodiesel CRM's, developed by Inmetro and NIST, were included. The agreement of results provided by participating laboratories, and the consensus related to reference values, were perceived as satisfactory. A clear demand was expressed for RMs of biodiesel derived not only from one feedstock but from the various ones. This fact happens due to the considerable difference in chemical and physical properties among biodiesels deriving from the diverse sources, which make harder the accommodation of all aspects relevant in quality control and the validation of analytical methods.

This project concluded that this was a positive example of collaboration among metrology institutes and useful exercise for establishing a common approach to the production of biofuels reference materials. However, for several specifications, the assignment of an SI traceable value still need further research due to the complexity of the material.

5. Conclusion

The use of fuels derived from renewable sources, like crops, microorganisms or animal derivatives, bring several advantages related to the environment, economy and the fewer dependence on the main fossil energy source, petroleum. Concerning environmental aspects the main fact that has been constantly emphasized, and actually is one the greatest advantage of using biofuels, is related to the reduction of greenhouse gases exhausts. Since biofuels are derived from renewable sources, the burning of biofuels in internal combustion engines linked to the renovation of those sources which biofuels are derived from, allows the establishment of CO₂ recycling in environment. Moreover, biodiesel production, especially in Brazil, has presented a great social advantage, seen that small farmers are encouraged to produce and furnish the raw material, which in turn stimulate the local economy of small cities and create new job positions.

From all the possible and already studied biodiesel sources, soybean presents great prominence. Soybean is a widespread culture, adapted to cultivation in several climates and countries, there is cultivation technology available and the logistics chain is well established. Otherwise, some aspects related to biodiesel production, economy, supplying and technical-economic availability need more profound studies. Literature points that a change in fatty acids profile of some feedstocks, using biologically modified species, in order to attend both human consumption and its utilization as fuel, would be one the most appropriate alternative for biodiesel replaces efficiently petrodiesel. Other aspect to be considered regards the byproduct glycerine recycling, as transforming it into products with commercial interest. Other point to be addressed is the gradual substitution of feedstocks that today are commodities (like soybean) for alternative raw materials that have less commercial importance, as macauba (*Acrocomia aculeata*) and jatropha (*Jatropha curcas*). This substitution would undervalue biodiesel production costs. Biodiesel quality control has also crucial importance. Concerning this subject there is a great need for the development of robust and specific analytical methods, since the existing ones are adapted from the petrodiesel quality control. Moreover, in order to ensure biodiesel quality, although some efforts have been done, only two certified reference materials (soybean and animal fat + soybean based ones) are available. Another challenge concerns the development or enhancement for getting more efficient biodiesel production processes, seen that the actual ones, like those employing homogeneous or heterogeneous catalysis, still present several drawbacks.

6. References

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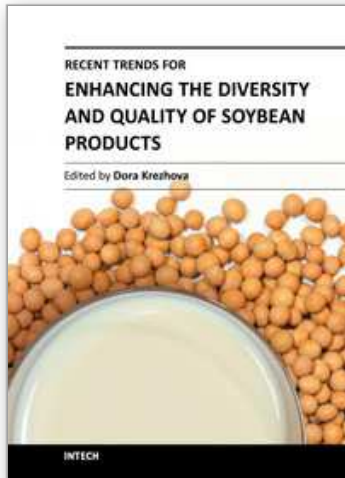
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