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Gas chromatographic analysis of free steroids in biodiesel

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HIGHLIGHTS

- Ten biodiesel types from various raw materials were studied in composition free steroids.
- Contents of steroids present in biodiesel determined by GC-FID showed great variability among them.
- Results obtained by GC–MS were used in identification of steroids quantified.
- Types of steroids present in biodiesel and its own peculiar distribution showed that some steroids were exclusive.
- Composition of steroids in this study allows the identification of different types of biodiesel in a blend them.

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ABSTRACT

This study aimed to extract, characterize and quantify free steroids present on various types of methyl biodiesel produced in the laboratory from different raw materials and a special type of commercial biodiesel. Steroid extraction was performed by sample saponification followed by solvent extraction of insaponificable. Steroid characterization was obtained by gas chromatograph equipped with a flame ionization detector (GC-FID) and by gas chromatograph coupled with a mass spectrometer (GC–MS); steroid quantification was performed by GC-FID. Several different free steroids found present in the biodiesel types were analyzed; some of them were identified as exclusive to certain biodiesel types. It was possible, based on the present free steroid profile analysis methodology, to identify the origin of a commercial biodiesel. Analyses had show that steroid content in biodiesel types ranged from 610 mg kg^{-1} in the babassu biodiesel to 3250 mg kg^{-1} in the crambe biodiesel.

1. Introduction

Brazil's potential for biodiesel production from various fatty raw materials has expanded throughout the country and over the years a number of vegetable and animal oils and fats have been cited as potential sources [1]. Given this great diversity in raw materials, it is possible to produce biodiesel with different specifications, which are monitored using methods defined in legislation and approved by the ANP (National Agency of Petroleum, Natural Gas and Biofuels) [2].

The continuous rise of biodiesel percentages added to diesel provides evidence of the success of Brazil's National Program of Biodiesel Production and Use (PNPB) and the country's accumulated experience in large-scale production and use of biofuels [2]. However, aspects pertaining to quality control still cause objection as regards the program's full success. This is the reason why controlling the quality of biodiesel and its blends with diesel is a critical step in ensuring the reliability of this biofuel [3].

To succeed in biodiesel quality is of paramount importance to know the origin of the fatty raw materials used in its production, since different specifications are obtained by different raw materials. Steroid composition has been used to characterize oils or fats [4–6]. It is also possible to characterize biodiesel produced by various fatty raw materials because reaction conditions of biodiesel production from oils and fats are not capable of eliminating these constituents from the biofuel matrix [7,8].

Steroids are lipids in order of ppm found in oils and fats known particularly for their 'fingerprint' in identifying these oils and fats [9]. Depending on the source of oils or fats under analysis, steroid content may vary, but rarely among types and their relative proportions [10]. Steroids may be distributed in free form (S) or conjugated form (ES, ASG, SG,) [11]. The present paper concerns a free forms distribution profile study.

Some biodiesel components are known to produce sediments. Previous studies have confirmed the presence of steroids in sediment formation [7,8,12,13]. These steroids may become insoluble

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in biodiesel due to medium polarity factors and to their considerable concentration in the biofuel [14].

In this sense, this study analyzes free steroids distributed in methyl biodiesel produced from various fatty raw materials. They are sought to obtain qualitative and quantitative data that may also help to characterize the biodiesel origin.

2. Materials and methods

2.1. Methyl biodiesel

Methyl biodiesel from various fatty raw materials, such as babassu, canola, cotton, crambe, palm, palm kernel, peanut, soybean, sunflower and tallow, were analyzed in laboratory according to procedure developed by Prados et al. [15].

2.2. Analysis free steroids

All experimental procedure for analysis of free steroids in biodiesel by gas chromatograph equipped with a flame ionization detector (GC-FID) and by gas chromatograph equipped with a mass spectrometer (GC–MS) was based on the procedure developed by Bezerra et al. [9] for analysis of steroids in vegetable oils.

2.3. Fortification recovery test

Cholesterol and β-sitosterol standard solutions at 10, 1000, and 2500 mg kg^{-1} concentrations were added to soybean biodiesel for fortification recovery testing. Before extracting steroids, the biodiesel was fortified and injected into GC-FID for analysis by Bezerra and Antoniosi Filho [9]. The analysis of each fortification sample was carried out in triplicate. Recoveries were calculated from the peak areas of test samples and compared with those of blank sample.

2.4. Analysis of commercial biodiesel

A Brazilian commercial biodiesel was subjected to experimental procedure of analysis of free steroids in biodiesel, in order to identify the raw materials used on its production.

3. Results and discussion

3.1. Fortification recovery test

The values obtained by recovery test for fortified β-sitosterol and cholesterol ranged from 90.31% to 95.76% and from 89.90% to 102.80%, respectively (Table 1). The results showed reasonable accuracy for method proposed. Relative standard deviation ranged from 8.65% to 10.64% for β-sitosterol and from 6.28% to 11.24% for cholesterol (Table 1), presenting excellent results as regards the method's precision. These results show that the method is good enough to quantify free steroids in biodiesel.

3.2. Qualitative analysis of free steroids

Through the GC-FID analysis, it was possible to identify the elution times of steroids found in various biodiesel types analyzed, as well as the elution times related to the internal standard (Table 2). Results show that all steroids eluted in times ranging from 18.71 min to 28.20 min. The elution time obtained for the internal standard betulin was 29.44 min. Results obtained by GC–MS were also used in identification of steroids. Table 2 lists the main fragmentation patterns obtained free steroids identified in all biodiesel.

Table 1
Results fortification recovery test.

Steroid	Spiked amount	*n*	Recovery (%)[a]	RSD (%)[b]
β-Sitosterol	10 mg kg^{-1}	3	93.66	8.65
	1000 mg kg^{-1}	3	90.31	9.91
	2500 mg kg^{-1}	3	95.76	10.64
Cholesterol	10 mg kg^{-1}	3	89.90	6.28
	1000 mg kg^{-1}	3	94.71	8.39
	2500 mg kg^{-1}	3	102.80	11.24

[a] Mean recovery.
[b] Relative standard deviation.

The presence of β-sitosterol, campesterol, brassicasterol, cholesterol, and stigmasterol was identified by elution times and by mass spectrometry results, obtained through the analyses of reference standards for each of these steroids. Steroids St. 1–St. 16 were identified by elution time and mass spectrometry analysis obtained by previous studies, particularly that of Bezerra and Antoniosi Filho [9]. Steroids St. 17–St. 21 were identified by GC–MS (Fig. 1), and their elution times characteristic identified by GC-FID (Table 2).

The fragmentation observed in the free steroids (Table 2) makes it possible to generate ions useful in locating substituents present in the ABCD-ring (Fig. 2), which forms steroids' basic structure.

In most cases, the number of double bonds found in the ABCD-ring after loss of the entire derivative group and loss of the side-chain may be predicted via mass spectra. In case of steroids with double bond in the side-chain, the loss of this chain is often accompanied by the loss of two D-ring's hydrogens [16,17].

As shown in Table 2, derivatized steroid easily loses the fragment M^+-90 by ionization that represents the loss of all derivatized structure linked to the steroid molecule. Other fragment formed in steroids is fragment M^+-105 that represents the loss of an entire derivatized along with a methyl side chain.

Mass spectra of Δ^5 steroids (which show unsaturation between carbons 5 and 6 at B-ring) reveal an intense peak, normally the base peak, at *m*/*z* 129, accompanied by ion M^+-129. β-sitosterol, brassicasterol, and cholesterol are examples of such steroids. An intense fragment ion is usually formed by loss of the entire side-chain; a fragment ion in this loss is normally represented by peak *m*/*z* 255. Nearly all steroids identified in this study showed this fragment, with the exception of steroids St. 1, St. 3, St. 7, and St. 19.

The fragment of side-chain *m*/*z* 213 was also selected as a peak of significant importance in steroids' identification, which represents D-ring cleavage. Another characteristic fragment, related to the cleavage of C- and D-rings, that was found in some of the identified steroids is *m*/*z* 173. Fragment *m*/*z* 73 [$Si(CH_3)_3$] is common in steroids derived by TMS substituents, and virtually all identified steroids showed this fragment ion.

3.3. Quantitative analysis of free steroids

The present study offers a new perspective in biodiesel identification, in that it brings forth the analysis of free steroids in addition to the analysis steroids in of oils and fats, as has been frequent in the literature. There was considerable diversity of biodiesel types under investigation, as well as a large number of steroids identified in each of them. Quantitative data obtained for steroid content in biodiesel (mg kg^{-1}) via GC-FID analysis are shown in Table 3.

β-Sitosterol was the predominant steroid in all oleaginous based biodiesel types, with over 50% in mass. Other important steroids identified in oleaginous are campesterol, stigmasterol, and St. 1. These data are in accordance with results described in the literature as regards oils and fats [4,6,18].