Quantification of Soybean Oil Ethanolysis with ^{1}H NMR

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ABSTRACT: In this study, proton NMR spectroscopy (200 MHz) was used for quantifying the content of ethyl esters in known mixtures of soybean oil and ethyl soyate (biodiesel). For this purpose, the peak areas of ester ethoxy and glycerol methylenic peaks in the region of 4.05–4.40 ppm were measured and a calibration plot of the respective peak areas vs. the known composition of the oil/ethyl ester mixtures was used. The transesterification values determined in this way were compared with viscosity and total glycerol determinations and a good correlation was obtained. Therefore, for routine analysis, the conversion (in %) of oil to ethyl esters was determined. The methodology presented in this work proved to be quicker and simpler than others reported in the literature, such as GC and/or HPLC.

Paper no. J10914 in *JAOCS 81*, 1111–1114 (December 2004).

KEY WORDS: Biodiesel, ethyl esters, ^{1}H NMR spectroscopy, soybean oil, transesterification.

Methyl and ethyl esters derived from vegetable oils are receiving considerable attention as alternative diesel engine fuels since they have fuel characteristics similar to petrodiesel and can be used in compression–ignition engines without modification (1,2). When used as fuels, they are typically referred to as biodiesel. Numerous studies have shown that most of the exhaust emissions encountered with petrodiesel are reduced by using biodiesel except for nitrogen oxides NO_x (3).

Transesterification is the process of producing a reaction between a TAG and an alcohol in the presence of a catalyst to produce glycerol and FA esters. The M.W. of a typical ester molecule is roughly one-third that of a typical TAG molecule; therefore, the ester has a much lower viscosity (4,5).

One main problem encountered in the study of or industrial applications of transesterification processes for vegetable oils is how to measure the methyl and ethyl ester content (6). NMR spectroscopy has become a prominent method for the determination of organic structures. The formation of FAME by transesterification with methanol can be monitored by ^{1}H NMR. NMR equipment, however, is expensive and accuracy of quantification can be an issue (3). In the literature there are several reports on the analysis of oils by NMR. The percentages of the FA with zero, one, two, or three double bonds in animal or vegetable oils have been determined by ^{13}C NMR (7). Olefinic carbon, n-3 methylene carbon, allylic carbon, and β-methylenic carbon from carbonyl carbon have been used to calculate the $C_{18:3}$, $C_{18:2}$, $C_{18:1}$, and $C_{18:0}$ FA compositions, respectively. Furthermore, the quantification of n-3 PUFA in fish lipids by ^{1}H NMR also has been reported, but other FA were not determined (7). Recently, fiber-optic NIR spectroscopy was used to monitor the methanolysis of a vegetable oil (soybean oil) to methyl esters, and the results correlated with ^{1}H NMR spectroscopy (8).

Commercial biodiesel oil is usually obtained by a transesterification of a fat or oil with methanol, but recently ethanol has been used by several researchers, mainly in investigations using enzymatic processes (9–11).

In the quantification of the ethyl esters using low-frequency ^{1}H NMR spectroscopy (200 MHz), there is a superimposition of ester ethoxy and glycerol methylenic hydrogen of the pure oil in the region of 4.05–4.40 ppm. When conversion is complete, however, this superimposition of proton signals is not important because the oil TAG are transformed into ethyl esters and the glycerol methylenic protons are not observed. When the conversion is partial, however, there is superimposition of proton signals that make it impossible to relate the proton areas as carried out by Gelbard *et al.* for methyl esters (3).

In our experiments the spectra of pure vegetable oil showed eight groups of signals, as described in literature (7,12). These signals were assigned as olefinic hydrogens; others, as the methylene hydrogen in the glycerol moiety; two methylene hydrogens in the glycerol moiety; diallyl methylene hydrogens; three CH_2 groups α to ester carbonyls; CH_2 groups attached to saturated carbons and double-bonded carbon atoms; CH_2 groups attached to saturated carbon atoms; CH_2 groups bonded to two saturated carbon atoms; and three terminal methyl groups.

The aim of our research was therefore to monitor and quantify the content (in %) of the ethyl esters in known mixtures of soybean oil and ethyl soyate (biodiesel), using ^{1}H NMR spectroscopy. The obtained results were also compared with viscosity and total glycerol and successfully applied to an enzymatic ethyl transesterification of soybean oil.

EXPERIMENTAL PROCEDURES

The soybean oils used for transesterification reactions were refined, edible-grade oils (Soya™), exhibiting an iodine number of 128.2 (Wijs) and a FFA content of 0.044% (oleic acid). Ethanol, HPLC grade, was used without further purification.

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Reagent-grade sodium hydroxide was used as catalyst. The viscosity was measured using a Brookfield viscometer (model LVDVIIICP CP 42), and density values were measured with the use of pycnometer and analytical balance both at 25°C. ^{1}H NMR analyses were performed on a Bruker Advance 200 MHz Spectrometer using $CDCl_3$ as solvent and tetramethylsilane as the internal standard.

The ethyl esters of soy oil TAG were prepared using anhydrous ethanol and sodium hydroxide according to Freedman (13). A general procedure for the transesterification was as follows: 1 g of NaOH was dissolved in 50 mL ethanol for 24 h and added to 100 mL soybean oil, and the mixture was gently heated for 8 h. After cooling, the upper layer was recovered, neutralized with glacial acetic acid, washed twice with brine to ensure complete removal of glycerol, dried over anhydrous magnesium sulfate, and finally filtered in a chromatography column with activated granular bauxite to remove the MAG and DAG (14), alternated between layers of powdered starch (15). To construct the calibration curve, 13 soy oil/ethyl ester mixtures (in percentages, 100:0, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80,10:90, 5:95, and 0:100 vol/vol) were prepared. The samples were analyzed by ^{1}H NMR over the region of 4.05–4.40 ppm, and for each one the area relation (A_1/A_2) was calculated. By plotting A_1/A_2 values vs. oil/ethyl ester percentage, a calibration curve was obtained and used for the quantification of soybean oil ethanolysis.

The content (%) of the ethyl esters in the mixtures with soybean oil also was measured according to the AOCS. Official Method Ca 14-56 for total, free, and combined glycerol (iodometric-periodic acid method (16). This method determines total glycerol by hydrolysis of the oil and reaction of the liberated glycerol with periodic acid. The resulting compound was titrated with normalized sodium thiosulfate (0.1 N) solution using a starch indicator. The extent of the reaction was calculated by computing the amount of glycerol in the original oil and subtracting from the total glycerol measured in the washed ester using Method Ca 14-56. This difference was stated as a percentage of the original total glycerol amount (17). The content (%) of the ethyl esters in the mixtures (compositions cited above) also was measured by applying a simple correlation with viscosity, according to de Filippis *et al.* (6). Viscosity values were plotted vs. known composition of oil/ethyl esters (cited above), which gave a linear correlation coefficient of 0.9981 (Fig. 1).

RESULTS AND DISCUSSION

A transesterification reaction consists of a number of consecutive, reversible reactions, the first step being the conversion of TAG to DAG, followed by the conversion of TAG to MAG and glycerol, yielding a single ethyl ester molecule at each step. After transesterification of the soybean oil and purification of the ethyl esters, a decrease in viscosity, density, and total glycerol (changing from 52.64 to 6.12 mPa·s, 0.9260 to 0.8810 g/cm^3, and 11.4 to 0.2%, respectively) was observed. The obtained values were in agreement with those previously reported for ethyl esters of soy oil (6).

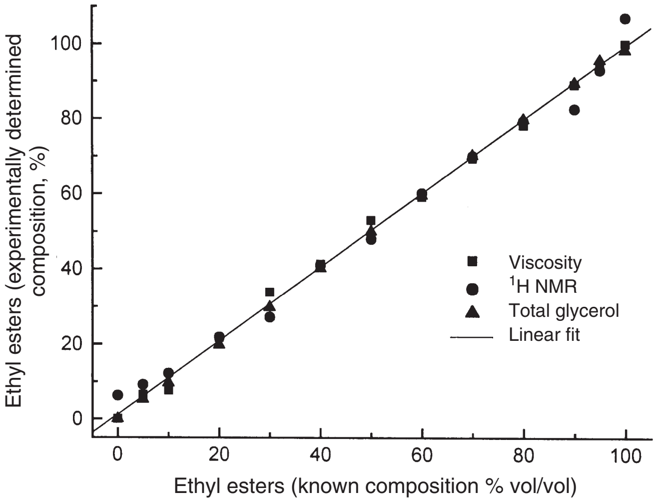

FIG. 1. Correlation between ethyl ester content in oil/ester mixtures of known composition and the experimentally determined obtained with ^{1}H NMR spectroscopy and measurements of viscosity and total glycerol.

Figures 2A, 2B, and 2C show a full-range NMR spectrum of pure soybean oil, pure ethyl esters, and expanded signals in the region 4.05–4.40 ppm for known compositions of oil/ethyl esters mixtures, respectively. Figures 2A and 2B were analyzed as described in the literature (7,12).

In the region where there is normally a superimposition of the relevant esters (quartet) and oil (multiplet) signals, it was observed that the quartet had a definite signal in pure esters (Figs. 2B, C6 in 2C). Figure 3 shows an expanded ^{1}H NMR spectrum in the region of 4.05–4.50 ppm, for a 50% oil/ethyl ester mixture.

Values of 0 and 1, used to construct the calibration curve, are defined as follows: When oil conversion into the ester is complete, the area relation is zero (0), and for pure oil it is one (1). As shown in Figure 2A and C1 in 2C, the double doublet areas for the glycerol methylene hydrogen are equivalent.

A calibration curve was obtained by plotting A_1/A_2 vs. known oil/ethyl ester composition (0–100% vol/vol) by plotting a median of three experimental points for each mixture. The term A_1 corresponds to the area representing glycerol hydrogen atoms, and term A_2 corresponds to the area of the ethoxy quartet of ethyl esters with glycerol peaks. A correlation coefficient of 0.9911 was obtained.

This calibration curve was successfully used to monitor enzymatic transesterification reactions of soybean oils with ethanol. By using Lipozyme RM IM (an immobilized lipase from *Mucor miehei* [5–6 BAUN/g] from Novozymes (Bagsvaerd, Denmark), the conversions in ethyl esters after 12 h at 35°C were 98, 65, and 35% using refined soybean oil, degummed, and discarded oil from a multiple frying process, respectively. Similar conversions were obtained when free Lipolase 100 L [a lipase from *Thermomyces lanuginosus* (100 KLU/ g) from Novozymes] was used as biocatalyst (15,18).

For quantification of ethyl esters in oil/ester mixtures, a previous purification process is not necessary and only a small amount of sample is required. However, by using ^{1}H NMR, it is not possible to detect MAG and/or DAG with this low-frequency spectrometer.

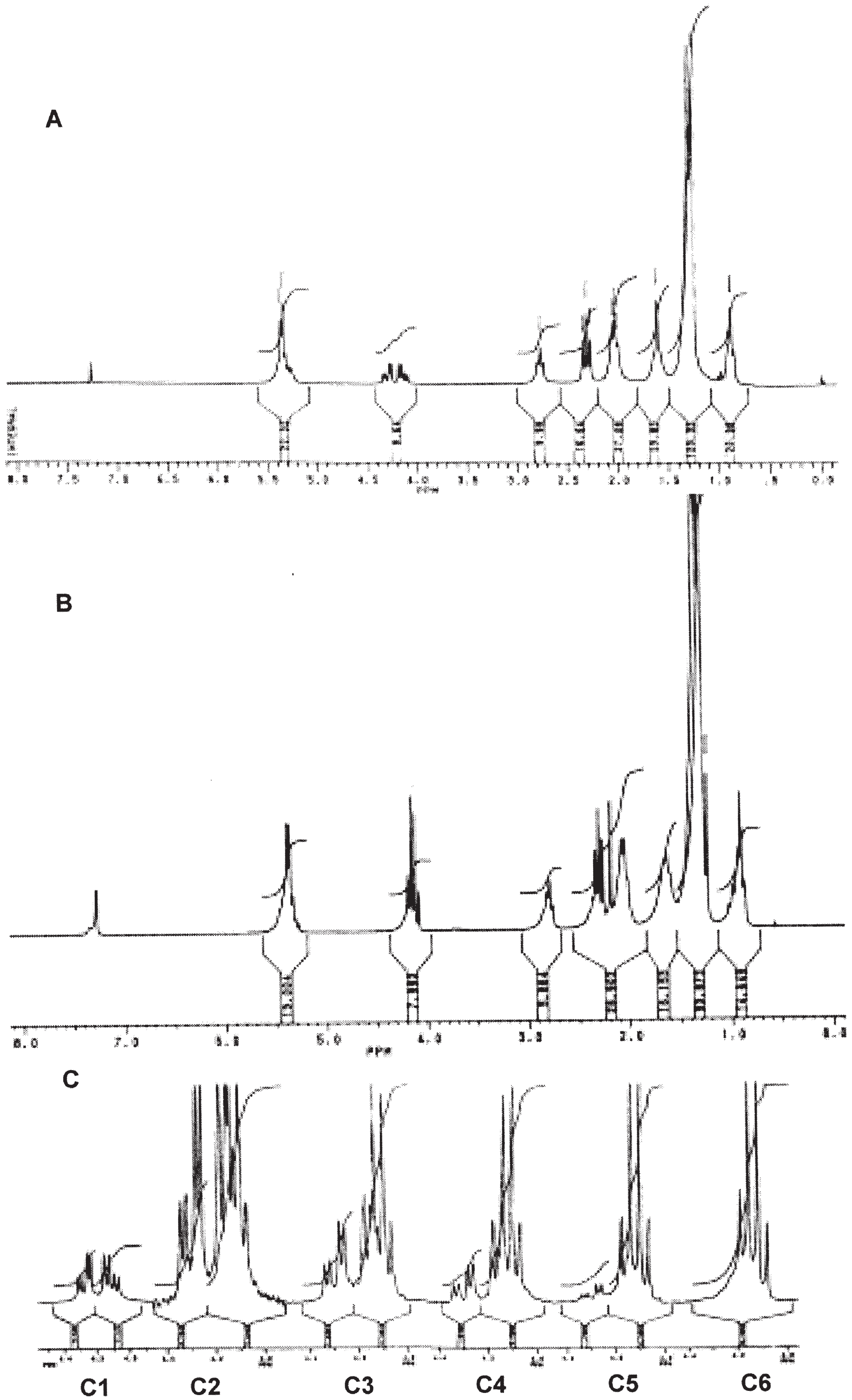

FIG. 2. ^{1}H NMR spectra: (A) pure soybean oil; (B) pure ethyl esters; (C) expanded signals over the region 4.05–4.40 ppm [C1, pure soybean oil; signal of oil/ethyl ester mixtures in known compositions of C2 (20:80), C3 (40:60), C4 (60:40), C5 (80:20% vol/vol); C6, pure ethyl esters], spectra collected at 200 MHz, $CDCl_3$.

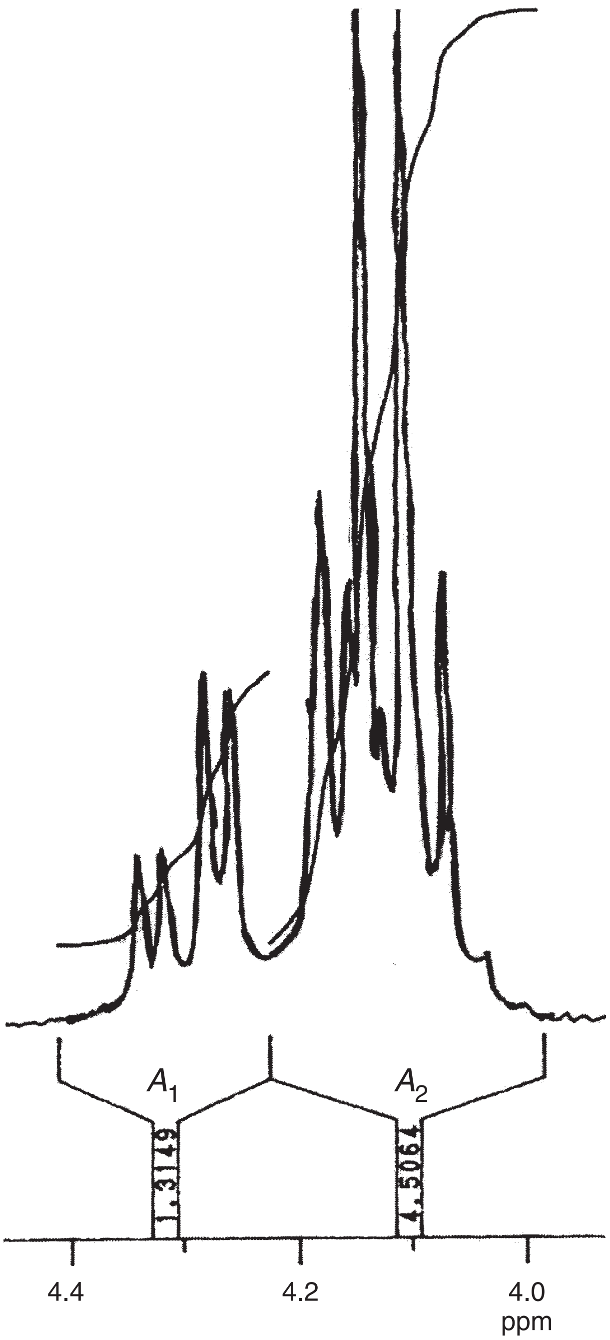

FIG. 3. Region in the ^{1}H NMR spectrum of a 50% oil/ethyl ester mixture (50:50 vol/vol), showing the signal superimposition at 4.05–4.40 ppm. A_1, the portion of glycerol hydrogen signals not superimposed by signals of ethyl ester, and A_2, signals of ethoxy quartet with glycerol peaks (200 MHz, $CDCl_3$).

Figure 1 shows that there is a good correlation in the known ethyl ester content from soybean oil ethyl transesterification reactions as determined by ^{1}H NMR and by viscosity techniques and total glycerol. These results show the feasibility and applicability of this method for routine analyses.

ACKNOWLEDGMENTS

We are grateful to the UFSC, to Centro Federal de Educação Tecnológica do Paraná (CEFET/PR/Brazil), to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Brazil), and to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brazil).

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[Received August 10, 2004; accepted November 26, 2004]