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Análise Quantitativa do Óleo de Tungue (*Aleuritis fordii*) por Espectroscopia de RMN de ^{1}H

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Tung Oil (*Aleuritis fordii*) Quantitative Analysis by ^{1}H-NMR Spectroscopy

Abstract: This work describes Equations derived from ^{1}H NMR integrated spectra data that enabled us to access several Tung oil quality indicators. Some of these useful quality indicators are laborious and time consuming when obtained by conventional analysis, but easily obtained through ^{1}H NMR data. Tung oil analysis performed in such way showed average molecular weight ranging 842.0-886.0 g/mol, iodine 222.0-228.0 gI_2/100g, saponification values 191.0-200.0 mg KOH/g, saturated fatty acid 2.0-7.0%, unsaturated fatty acid 93.0-98.0%, ratio of olefinic to aliphatic hydrogens 1.74-1.84, alpha-eleostearic 72.7-81.6%, linolenic 0.0%, linoleic 1.6-8.7% and oleic acids 8.0-15.0%. These results are in agreement with earlier reports regarding fatty acid contents in tung oil and indicated that ^{1}H NMR spectroscopy is a very useful tool in oil analysis, yielding results similar or superior to those obtained by classical analytical methods.

Keywords: ^{1}H-NMR spectroscopy; alpha-eleostearic acid; tung oil quality indicators.

Resumo

Este trabalho descreve as equações derivadas da integração dos dados do espectro de RMN de ^{1}H que nos permitiram acessar vários indicadores de qualidade do óleo de tungue. Alguns desses úteis indicadores de qualidade são laboriosos e consomem tempo quando obtidos por análises convencionais, mas facilmente acessíveis por dados de RMN de ^{1}H. As análises realizadas dessa maneira mostraram que o óleo de tungue apresentou peso molecular médio em cerca de 842,0-886,0 g/mol, iodo 222,0-228,0 gI_2/100g, índice de saponificação 191,0-200,0 mg KOH/g, ácidos graxos saturados 2,0-7,0%, ácidos graxos insaturados 93,0-98,0%, proporção de hidrogênios olefínicos por alifáticos 1,74-1,84, ácidos alfa-eleosteárico 72,7-81,6%, linolênico 0,0%, linoléico 1,6-8,7% e oléico 8,0-15,0%. Estes resultados estão de acordo com informações anteriores em relação aos ácidos graxos contidos no óleo de tungue e indicaram que a espectroscopia de RMN de ^{1}H é uma ferramenta muito útil em análises de óleos, produzindo resultados semelhantes ou superiores aos obtidos por métodos clássicos de análise.

Palavras-chave: Espectroscopia de RMN de ^{1}H; ácido alfa-eleosteárico; indicadores de qualidade do óleo de tungue.

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Análise Quantitativa do Óleo de Tungue (*Aleuritis fordii*) por Espectroscopia de RMN de ^{1}H

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1. Introduction

Much of the tung tree (*Aleuritis fordii*) land area in Brazil is located in the southern region mainly on Parana and Rio Grande do Sul states.[1] The tung oil, obtained from the seeds of the tung tree, is a high quality industrial drying oil and it is primarily used as wood varnish and in formulations of inks, coatings and resins. Its unique drying qualities are attributed to its fatty acid composition, dominated by approximately 80% alpha-eleostearic acid, a conjugated trienoic fatty acid.[2]

Unlike most kinds of vegetable oils that contain common fatty acids, e.g., palmitic (16:0 hexadecanoic), stearic (18:0 octadecanoic), oleic ($18:1^{9Z}$ octadecenoic), linoleic ($18:2^{9Z,12Z}$ octadecadienoic) and linolenic ($18:3^{9Z,12Z,15Z}$ octadecatrienoic) acids,[4] the major fatty acid in tung oil is the alpha-eleostearic acid (αESA, $18:3^{9Z,11E,13E}$ octadecatrienoic) an ω-5 conjugated linolenic acid (CLnA), containing three conjugated carbon-carbon double bonds.[5] Both, αESA and its isomer, minor component beta-eleostearic acid (βESA, $18:3^{9E,11E,13E}$ octadecatrienoic) can be identified as markers of tung oil presence in samples like aged material exudation.[6] Beta-eleostearic acid ($18:3^{9E,11E,13E}$) can be also derived from alpha-eleostearic acid ($18:3^{9Z,11E,13E}$) through thermal isomerization.

CLnA are a group of positional and geometric isomers of octadecatrienoic acid (C18:3) that contain three conjugated double bonds, primarily in positions $\Delta^{9,11,13}$ or $\Delta^{8,10,12}$ and exist in both *Z* and *E* geometrical isomers. In nature, CLnA and conjugated linoleic acid (CLA) are not very common in yeasts, bacteria, fish oil or animal fats, but are found in abundance in many seed oils as

conjugated C18 dienoic, trienoic and tetraenoic fatty acids.[7]

Seven species of conjugated trienoic fatty acids (CTFAs) are commonly known: Punicic acid (18:3^{9Z,11E,13Z}) is found in pomegranate (*Punica granatum L.*) and the seeds of *Trichosanthes kiirilowii*; Jacaric acid (18:3^{8Z,10E,12Z}) is found in seeds of *Jacaranda mimosifolia*; Alpha-eleostearic acid (18:3^{9Z,11E,13E}) is present in tung seed oil (*Aleuritis fordii*), manketti nut oil (*Ricinodendron rautanenii*) and in bitter gourd oil (*Momordica charantia* L.); Catalpic acid (18:3^{9E,11E,13Z}) is present in Catalpa seed (*Catalpa bignonoides* and *Catalpa ovata*); alpha-Calendic acid (18:3^{8E,10E,12Z}) and beta-calendic acid (18:3^{8E,10E,12E}) that are found in the seeds of pot marigold (*Calendula officinalis* L.) and beta-eleostearic acid (18:3^{9E,11E,13E}) is present in pomegranate, bitter gourd and catalpa.[7,8]

The economic importance of tung oil is related to vegetable oil production, through seed compression, whose residue contains high amounts of potassium (23.0%), calcium (9.5%) and magnesium (17.0%) being used in organic fertilization. This oil has several industrial applications, especially wood preservation, being a source of drying oil used in paints, varnishes and for different sorts of polymerization. Tung oil was used as an ingredient in historical conservation material. Wooden art objects were treated with tung and linseed oil mixtures for consolidation purposes.[6]

It has been reported that CLnA, like alpha-eleostearic acid, are potent growth suppressors of various human tumor cells, as leukemia cells and also demonstrate strong antioxidant activity in plasma lipid, lipoprotein and erythrocyte membrane peroxidations in rats. It is also known that biological activity varies with different CLnA isomers.[8-14]

Oils containing CLnAs are very important raw material in the manufacture of organic coatings and polymers, as conjugated unsaturation facilitates good polymerization and imparts adhesive properties when properly treated.[7] Heating or chemical treatment of pure tung oil will substantially increase the viscosity and film-forming quality of the product. The resulting oil will range in consistency from that of maple syrup to that of motor oil. Some brands include quick evaporating thinners to make it easier to work with and improve wood penetration. As the penetrating power of tung oil is excellent and it will adhere to porous minerals, it is often used by stonemasons on granite or marbled installed in kitchens, bathrooms, and other staining fluid environments.[6]

Tung oil composition and quality are usually analyzed by ultraviolet (UV) and infrared (IR) spectroscopy, gas chromatography (GC) and High-performance liquid chromatography (HPLC), but these techniques are unable to identify the location of C=C double bond precisely. Components can be identified by mass spectrometry (MS) performed with electron impact (EI), chemical ionization tandem mass spectrometry (CIMS/MS) in isobutene or by combination of gas chromatography with mass spectrometry (CGMS) and gas liquid chromatography with mass spectrometry (GLCMS). CG-MS is effective and accurate. Unequivocal identification can be achieved by nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR). Isomers characterization is achieved by assigning the C=C double bonds using CG-MS. Hydrogen and carbon atoms chemical shifts are assigned using ^{1}H-NMR, ^{13}C-NMR, homonuclear two-dimensional correlation spectra (^{1}H-^{1}H COSY) and heteronuclear two-dimensional correlation spectra (^{13}C-^{1}H COSY, HMQC, HMBC) and hydrogen-hydrogen coupling constants across the double bonds as determined by homonuclear decoupling selective technique.[3,8,15]

To provide a reference method to oil component identification, tung oil was examined in this study, both fresh and under heated extraction conditions, through ^{1}H-NMR spectroscopy, to perform its fatty acid composition and display an alternative method for its study.

2. Experimental

Tung oil extraction: tung oil samples extracted by pressing at room temperature (25°C) and heating below 115 and 135°C were supplied by IAPAR, Ponta Grossa-PR, Brazil. The seeds, also supplied by IAPAR, were dried (50°C, 8h), then pulverized in microprocessor and extracted with hexane at 70°C in Soxhlet extractor. The crude oil was obtained by removing the solvent in rotary evaporator. Throughout the extraction steps, seed and oil were protected from the light.

^{1}H-NMR conditions were as follows: solvent $CDCl_3$; room temperature; Varian Mercury-300 spectrometer; relaxation delay: 1.359 s; pulse: 45 degrees; acquisition time: 3.674 s; width: 4.498,4 Hz; repetition times: 16; ^{1}H nucleus observed at 300.058 MHz; line broad: 0.3 Hz; FT size: 65,536; total time: 1min22s. Simulated ^{1}H-NMR spectra were performed using ACD/ChemSketch NMR Processor Academic Edition 12.0 predictor program. Correlations were performed using the Microsoft Excel program. The simulated tung oil ^{1}H-NMR spectrum obtained and shown in figure 3, was very similar to the real spectra obtained from the tung oil samples (not shown). The integration values obtained from the real spectra were used to calculate several tung oil quality indicators (table 1) through the equations (1 - 13).

3. Results and Discussion

The tung oil fatty acid ^{1}H-NMR spectrum is well established in the literature.[15] The spectrum shows characteristic signals to olefinic and alkyl hydrogens. It is remarkable that the major component is alpha-eleostearic acid (71-82%) a CLnA marker responsible by its properties. This unsaturated fatty acid show peaks beyond 5.50 ppm (5.70 - 6.70 ppm), belonging to 5 olefinic hydrogen from its three conjugated carbon-carbon double bonds (18:3^{9Z,11E,13E} - Figure 1).[15]

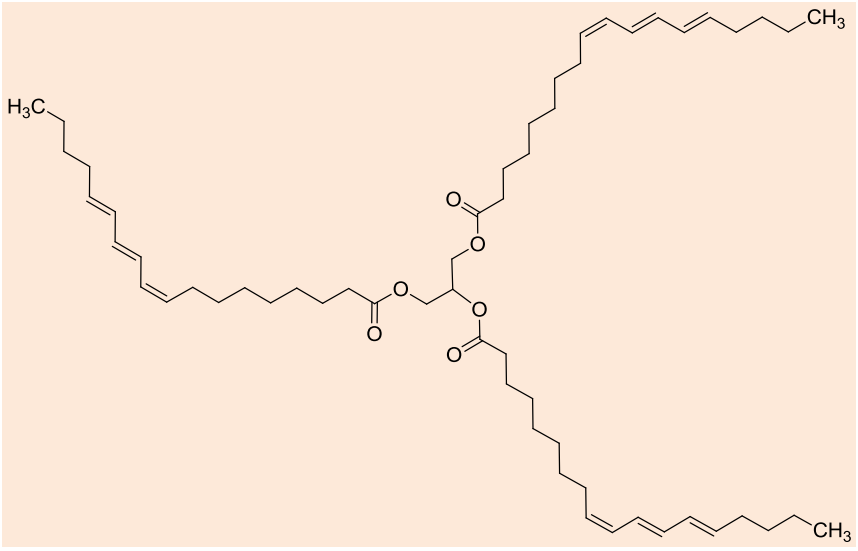

Figure 1.The alpha-eleostearic acid in triacylglyceride tung oil (18:3^{9Z,11E,13E})

A triacylglycerol (TAG) simulated ^{1}H-NMR spectrum of linseed and tung oil major components was performed as comparison analysis (Figures 2 and 3). In figures 2 and 3 the resemblance between both spectra is remarkable. At 5.50 ppm both showed the

same signal in accordance with the literature, showing characteristic peaks of olefinic (vinylic) and alkyl hydrogens. The peaks are as follows: hydrogen attached to olefinic carbon, appeared at 5.40-5.26 ppm (K); hydrogen belonging to methylene bis-allylic carbon at 2.90-2.70 ppm (G); alpha-carbonyl methylene at 2.35-2.25 ppm (F); allylic methylene at 2.10-1.90 ppm (E); beta-carbonyl methylene at 1.70-1.50 ppm (D). A methylene cluster was found at 1.40-1.15 ppm (C); methyl hydrogen of linolenic acid appeared at 0.98-0.93 ppm (B); other fatty acid methyl hydrogen were found at 0.90-0.80 ppm (A). Glycerol methylenes hydrogen appeared at 4.32-4.10 ppm as doublet of doublets (I, H), and its methylene hydrogen (J) at 5.26 ppm, superposed to olefinic hydrogens.[17,18]

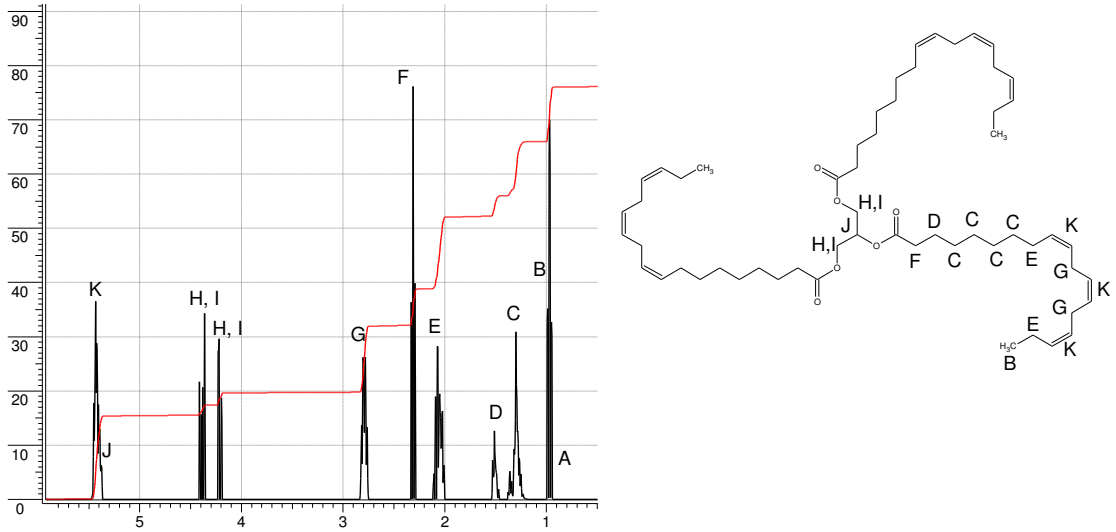

Figure 2. Simulated TAG ^{1}H-NMR spectra of 1,2,3-tris-gliceryl-linolenate (linseed oil)

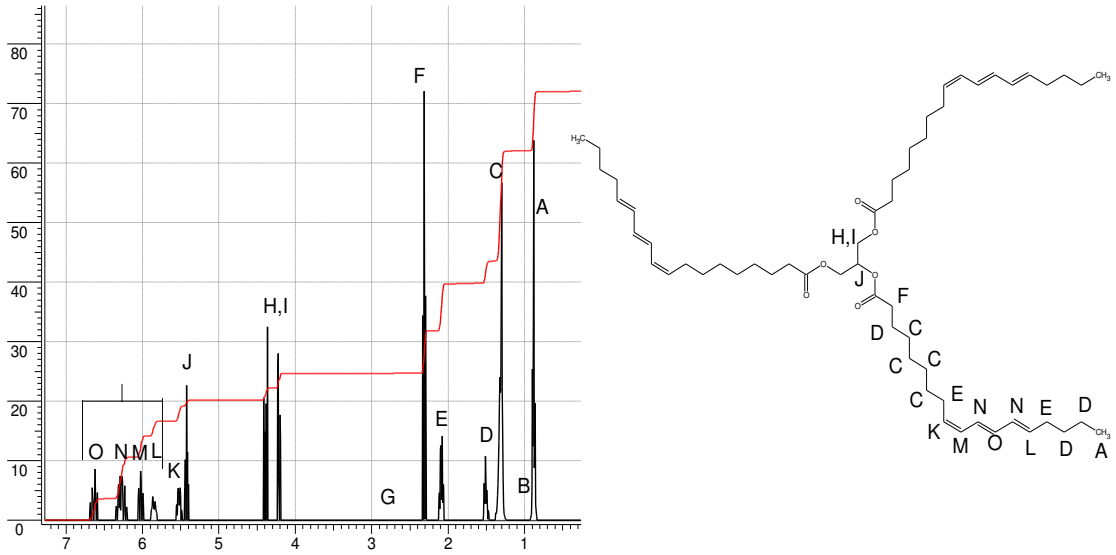

Figure 3. Simulated TAG ^{1}H-NMR spectra of 1,2,3-tris-gliceryl-α-eleostearate (tung oil)

3.1. Tung oil parameters determinations

The unsaturated fatty acids (UFA) profile was obtained as follows: The integration curve in figure 3 revealed that peaks beyond 5.50 ppm (5.70-6.70 ppm), signals L, M, N, O with intensity of 1:1:2:1 respectively, belong to 5 olefinic hydrogen from alpha-eleostearic acid ($18:3^{9Z,11E,13E}$). Their sixty olefinic hydrogen are found at 5.50 ppm as a cluster with different hydrogen from UFA like oleic, linoleic and linolenic acids, when present in TAG. Therefore, these 5 hydrogens (3 times in TAG) enabled us to determine the αESA (major component in tung oil) as conjugated linolenic acids (CLnA) directly from ^{1}H-NMR spectra data in a simple and easy way through Equation 1.

$$CLnA = \alpha ESA(\%) = \Sigma[L, M, N, O]/15 \quad \text{(Equation 1)}$$

Polyunsaturated fatty acid (PUFA) expressed as linolenic acid content ($C18:3^{9Z,12Z,15Z}$), can be determined through Equation 2 according to Guillén (2001).[17]

$$PUFA = Ln\ (\%) = B/[B+A] \quad \text{(Equation 2)}$$

Bis-allylic hydrogen (G) at 2.80-2.70 ppm is present in linolenic and linoleic acids. If the TAG acyl group is linolenic acid there are 4 bis-allylic hydrogens; if it is the linoleic acid, than there are 2 bis-allylic hydrogen. Thereupon, G = [4 Ln + 2 L]3 in TAG, and the diunsaturated fatty acid concentration (DUFA) can be determined as linoleic acid (L) through Equation 3 as described by Carneiro (2005).[19]

$$DUFA = L\ (\%) = [G/6] - 2\ Ln \quad \text{(Equation 3)}$$

Hydrogen (E) attached in allylic carbons at 2.10-1.90 ppm is present in alpha-eleostearic (αESA), linolenic (Ln), linoleic (L) and oleic (O) acids. Se the TAG acyl group is one of these compounds, there are 4 bis-allylic hydrogens for each acid. So, E = [4 αESA + 4 Ln + 4 L + 4 O]3 in TAG, and unsaturated fatty acid contents [UFA] can be determined through Equation 4.

$$UFA\ (\%) = E/12 = \Sigma\ [\alpha ESA + Ln + L + O] \quad \text{(Equation 4)}$$

This equation can be rewritten as Equation 5 by inserting linolenic and linoleic acid values, according to Equations 2-3 implying that monounsaturated fatty acid concentration (MUFA) can be determined as oleic acid through Equation 5.

$$MUFA = O\ (\%) = [E/12] - [G/6] + Ln - \alpha ESA \quad \text{(Equation 5)}$$

In figure 3 vinylic (olefinic) hydrogens can be determined by V = [K + L + M + N + O] integrated signals. Considering the 6 olefinic hydrogens in both linolenic and alpha-eleostearic acids, 4 hydrogens in linoleic acid and only 2 hydrogens in oleic acid, and therefore 3 times in TAG if some of these acids are present, vinylic (olefinic) hydrogen concentration can be determined through Equation 6

$$V/6 = 3\,\alpha ESA + 3\,Ln + 2L + O \qquad \text{(Equation 6)}$$

This equation can be rewritten as Equation 7 by inserting alpha-eleostearic, linolenic, linoleic and oleic acid values, according to Equation 1, 2, 3 and 5 implying that:

$$V = [4\alpha ESA/5] + [E/2] + G \qquad \text{(Equation 7)}$$

In figures 1 and 2, the sum of all hydrogen (A-K or A-O) performed 100.00%. Nevertheless to make calculations using Equations 1-7 previously shown, it is important to know the hydrogen real number belonging to each signal like V, E, G, αESA, that can only be obtained by dividing their values by the area of one hydrogen. Besides, all integrated values need to be correct by hydrogen area [A_{1H} = F/6] as described in this paper, or [A_{1H} = [I+H]/4] according to Guillén (2001).[17] Consequently the real number of vinylic (olefinic) hydrogen can also be determined through Equation 8, derived from Equation 7 by dividing it by the hydrogen area.

$$Vc = \{[\,24\alpha ESA/5] + 3E + 6G\}/F \qquad \text{(Equation 8)}$$

Saturated fatty acid is obtained as complement of 1 or 100% according to Equation 9.

Iodine values are determined through Equation 10, as previously related by Johnson and Shoolery (1962).[20]

$$SFA = 1\text{-}UFA \qquad \text{(Equation 9)}$$

$$IV = [12690.447\ V]/Mw \qquad \text{(Equation 10)}$$

Saponification values can be obtained through Equation 11, related by Carneiro (2005).[19]

As pointed out by previous works, Equation 12 can be used to calculate vegetable oils average molecular weight.[20; 19]

$$SV = -\,0.2353\ Mw + 398.44 \qquad \text{(Equation 11)}$$

$$Mw = 120.018 + 7.0135\ T_H + 6.006\ Vc \qquad \text{(Equation 12)}$$

Where T_H is the hydrogen total contents (%) obtained from the ^{1}H NMR data of integrated spectra, e.g., the sum of A-K or A-O hydrogen in figures 2 and 3 respectively, divided by the hydrogen area [$T_H = 100/A_{1H}$].[18] The hydrogen area is calculated from [$A_{1H} = F/6$].

The ratio of olefinic to aliphatic hydrogen was determined through Equation 13 according to Guillén (2001).[17]

$$R_{oa} = V/[A+B] \qquad \text{(Equation 13)}$$

These equations were used to calculate several tung oil quality parameters and its fatty acid content from ^{1}H NMR spectra data (table 1).

Table 1. Tung oil quality parameters and fatty acid content for the tung oil samples by ^{1}H NMR

Values	**Reference values ***	**^{1}H-NMR Extr. 25°C**	**^{1}H-NMR Extr. 70°C**	**^{1}H-NMR Extr. 115°C**	**^{1}H-NMR Extr.135°C**
Iodine index (g I_2/100g)	160.0-175.0	228.16	222.98	223.97	225.86
Saponification index (mg KOH/g)	189.0-195.0	191.69	194.44	200.30	196.78
Fatty acid content (%)					
Palmitic acid (16:0)	1.0-3.0	-**	-	-	-
Stearic acid (18:0)	1.0-3.0	-	-	-	-
ω-9 oleic acid (18:1^{9Z})	4.0-10.0	12.05	12.46	12.10	7.90
ω-6 linoleic acid (18:2^{9Z,12Z})	8.0-15.0	3.15	5.29	8.70	8.60
ω-3 linolenic acid (18:3^{9Z,12Z,15Z})	0.0-2.0	0.00	0.00	0.00	0.00
ω-5, α-eleostearic acid (18:3^{9Z,11E,13E})	71.0-82.0	81.64	76.95	72.70	76.40
SFA	5.0-15.0	3.16	5.30	6.50	7.10
UFA	85.0-95.0	96.84	94.70	93.50	92.90
M_w	-	878.63	866.98	842.08	857.04
R_{oa}	-	1.84	1.74	1.84	1.81

*AOCS, 2005.[16]

** - not determined

4. Conclusions

Tung oil quality parameters can be utilized in routine analysis as analytical alternative procedure to experimental AOCS methods, which are more laborious and time consuming. These results also showed the remarkable technical efficacy, quickness and precision besides that ^{1}H-NMR spectroscopy enable us to determine several oil quality indicators useful in oil analysis. Surely ^{1}H-NMR spectroscopic methods can be successfully applied to vegetable oils quality control.

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