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^{1}H NMR Characterization of Seed Oils from Rangpur Lime (*Citrus limonia)* and "Sicilian" Lemon (*Citrus limon*)

P. I. B. Carneiro*, S. Y. Reda, E. B. B. Carneiro
Universidade Estadual de Ponta Grossa, CP 992, Paraná, Brazil
pibc@uepg.br

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Abstract: *The purpose of this study was to characterize the oil extracted from rangpur lime and "Sicilian" lemon seeds and to compare the parameters determined by* ^{1}H *NMR spectroscopy data and classical determinations. Several parameters of oil quality were determined from integrated* ^{1}H *NMR spectra and by classical methods: saponification, iodine and acidity values as well the triacylglycerol average molecular weight, total saturated fatty acid contents, degree of unsaturation (linolenic, linoleic and oleic acids contents) and the ratio of olefinic to aliphatic hydrogens, that indicate in a precise manner, the oxidation state of the vegetable oil. The parameters determined are in a good agreement with experimental data. The oils extracted from two sources showed similar chemical composition, high degree of unsaturation (71.8 and 73.0 %) and are comparable to the edible oils of a good quality. The* ^{1}H *NMR spectroscopy is very useful in food analysis, yielding results similar or superior to those obtained by classical procedures.*

Resumo: *O propósito deste estudo foi caracterizar os óleos extraídos das sementes dos limões rosa e siciliano e comparar os parâmetros determinados por espectroscopia de RMN de* ^{1}H *e por determinações clássicas. Diversos parâmetros de qualidade de óleos foram determinados a partir do espectro integrado de RMN de* ^{1}H *e por métodos clássicos: índices de saponificação, iodo e acidez, bem como o peso molecular médio do triacilglicerol, teor de ácidos graxos saturados, grau de insaturação (teores de ácidos linolênico, linoleico e oleico) e a razão entre os prótons olefínicos e alifáticos, que indica de modo preciso, o estado de oxidação do óleo vegetal. Os óleos extraídos das duas fontes apresentaram composição química semelhante, alto grau de insaturação (71,8 e 73,0 %) e são comparáveis aos óleos comestíveis de boa qualidade. A espectroscopia de RMN de* ^{1}H *é muito útil na análise de alimentos fornecendo resultados semelhantes ou superiores aos obtidos por métodos clássicos.*

Introduction

Nowadays, the analysis of vegetable oils for quality control is mainly focused on acidity, peroxide value, ultraviolet absorption, and chromatographic techniques like TLC, HPLC and GC.[1] These techniques are primarily used for quantitative measurement of known compounds, and, with these analytical criteria, different international regulations have been established to define oil genuineness and quality. One drawback to these procedures is that there are too many different assays to be applied to routine analysis. In addition, some of these methods require the isolation and analysis of minor compounds by means of procedures that are laborious and time-consuming. Therefore, is desirable to apply analytical techniques like ^{1}H NMR, which can produce results similar or superior to those obtained by the classic procedures.[2] The purpose of this study was to characterize the oil extracted from Rangpur lime and "Sicilian" lemon seeds and to compare the parameters determined by ^{1}H NMR spectroscopy data and classical determinations.

Experimental

Seed oils were obtained by Soxhlet extraction using hexane as solvent, during 8 hours under reflux followed by solvent removal. According to the AOCS official methods[3], saponification index

(AOCS-Cd 3-25), iodine (AOCS-Cd 1b-87) and acidity values (AOCS-Ca 5a-40) were determined, as well as the profile of the fatty acids content by gas chromatography (AOCS-Ce 1-62). Spectra data were recorded on a Varian Mercury-300 spectrometer, operating at 300 MHz at room temperature.[4] The samples concentration for the ^{1}H NMR analysis was about 10 % of oil in 0.7 mL of $CDCl_3$ containing a very small amount of TMS as internal reference in a 5 mm NMR tube. The spectrum was acquired by using 16K data point, spectral width 14 ppm, acquisition time 3.6 s, relaxation delay 1.3 s, pulse width 45º and 16 scans, and total time about 90 s.

Results and discussion

Triacylglycerols are the major components (ca. 95%) of vegetable oils and show hydrogen chemical shifts (Figure 1) well established in the literature.[2,4,5,6,7] The peaks in ^{1}H NMR spectrum for unsaturated fatty acids are those of the olefinic hydrogen at δ 5.40-5.26 ppm, which also include glycerol carbons. Hydrogen attached to methylene glycerol carbons appears at δ 4.32-4.10 ppm; to bis-allylic carbons at δ 2.80-2.70 ppm; to α-carbonyl methylene carbons at δ 2.35-2.20 ppm.

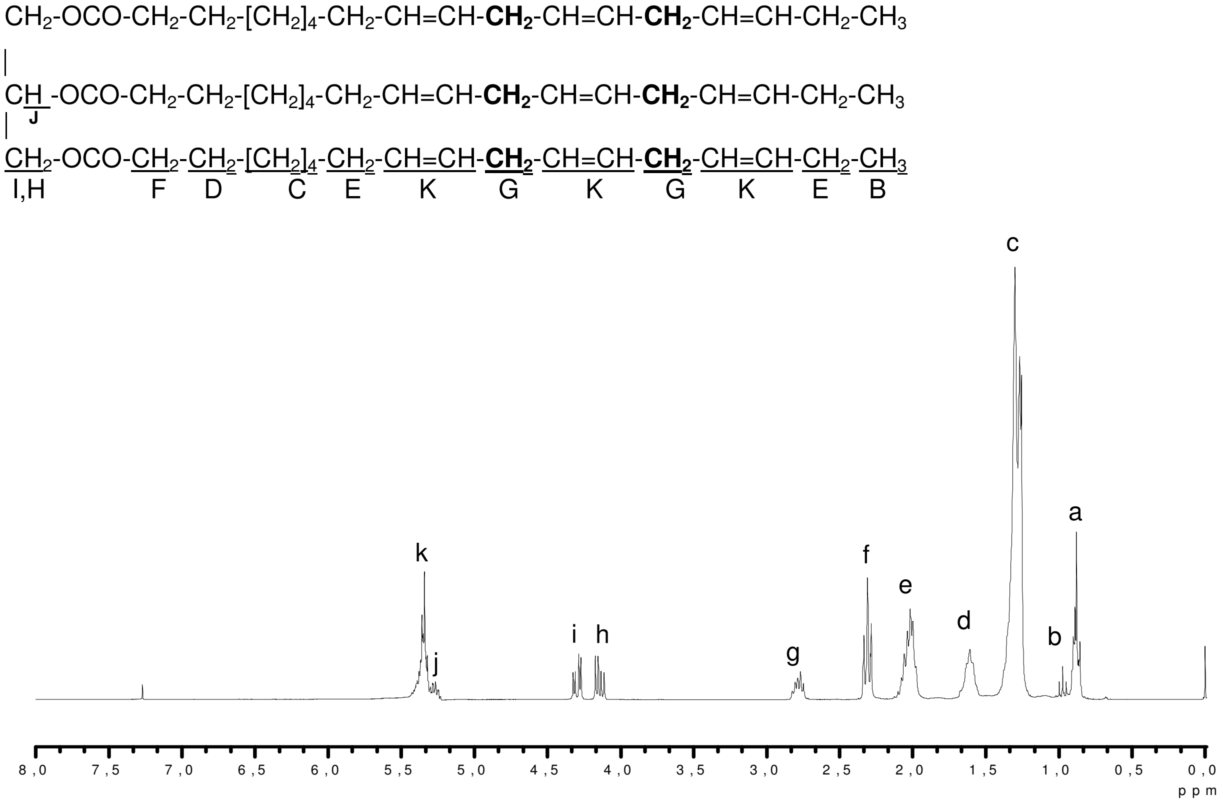

Figure 1. ^{1}H NMR spectrum of a triacylglycerol.

Hydrogen attached to allylic carbons at δ 2.10-1.90 ppm; to β-carbonyl methylene carbons at δ 1.70-1.50 ppm; for other fatty acid methylene carbons at δ 1.40-1.15 ppm; to methyl carbons of the linolenic acid at δ 0.95 ppm and at other methyl carbons of fatty acids at δ 0.88 ppm. The signal of terminal methyl groups can be used for determining the amount of linolenic acid (C18:3). With the proximity of the C15-C16 double bond in linolenic acid to the terminal CH_3, the signal of the terminal CH_3 is shifted downfield to approximately 0.95 ppm (anisotropic effects) and can be integrated separately from the signal of terminal CH_3 in the other fatty acid chains.

By using integrated signal of ^1H NMR spectra data of aliphatic and olefinic hydrogens, equations 1-10 were formulated and used to determine the average molecular weight, iodine, free acidity and saponification index, as well as saturated fatty acid amount, unsaturated fatty acid (UFA) or degree of unsaturation (DU), linolenic, linoleic, and oleic acid contents, and the ratio of olefinic to aliphatic values (R_{oa}), which indicate precisely the oxidation state of the vegetable oil.[8]

Parameters determination:

Triacylglycerol molecular weight:

$M_w = 119.70 + 7.036\ T + 5.983\ V$ (**Eq. 1**)

Where T is the total hydrogen content (%), and V the vinyl amount obtained from the integrated ^1H NMR spectra data.

Vinyl hydrogen:

$V = E/2 + G$ (**Eq. 2**)

Iodine index:

$I\ I = [126.91 \times 100\ V]/M_w$ (**Eq. 3**)

Saponification index:

$S\ I = -0.2358\ M_w + 398.42$ (**Eq. 4**)

$[r = 0.9998;\ r^2 = 0.9997;\ n = 5]$

Free acidity:

$FA = 3.0597\ [R_{oa}]^2 - 6.3181\ R_{oa} + 3.3381$ (**Eq. 5**)

$[r = 0.9878;\ r^2 = 0.975;\ n = 20]$

Ratio of olefinic to aliphatic:

$R_{oa} = V/[A + B]$ (**Eq. 6**)

Where B and A are the amount of hydrogen attached at the methyl groups in the linolenic and the other fatty acids, respectively.

Degree of unsaturation:

$D\ U = E/12$ (**Eq. 7**)

(unsaturated fatty acid)

Linolenic content:

$Ln = B/[B+A]$ (**Eq. 8**)

Linoleic content:

$L = G/6 - 2\ Ln$ (**Eq. 9**)

Oleic content:

$O = E/12 - G/6 + Ln$ (**Eq. 10**)

Equations 1, 2 and 3 were used as previously shown by Nathan[6] in 1982. Equations 4 and 5 were obtained from correlation analysis[8] between molecular weight (Eq. 1) and saponification index (experimental data); and free acidity (experimental data) with olefinic to aliphatic hydrogens ratio (Eq.

6). Eqs. 6 and 8 were used according to previous work by Guillén.[2] Eqs. 7, 9 and 10 were obtained as follows[8]: bis-allylic hydrogen (G) at δ 2.80-2.70 ppm is present in linolenic and linoleic acids. Whether the triacyl group in the triacylglycerol is linolenic acid, there are (3x4 H) 12 hydrogen bis-allylic; whether linoleic acid, then there are (3x2 H) 6 hydrogen bis-allylic. So,

$$G = 12\ Ln + 6\ L,$$
$$L = G/6 - 2\ Ln \qquad (\textbf{Eq. 9})$$

Hydrogen attached at allylic carbons in δ 2.10-1.90 ppm (E) is present in the linolenic (Ln), linoleic (L) and oleic (O) acids. The allylic hydrogen content of any of these acids are (3x4 H) 12. Thus,

$$E = 12\ Ln + 12\ L + 12\ O$$

This equation can be rewritten as Eq. 10 by inserting linolenic and linoleic acid values, according to Eqs. 8 and 9, implying that:

$$O = E/12 - G/6 + Ln \qquad (\textbf{Eq. 10})$$

Eq 7 was obtained by the sum of Eqs. 8-10. Table 1 shows the results of experimental and calculated values by ^{1}H NMR spectroscopy.

Table 1. Physicochemical characterization of Rangpur Lime (*Citrus limonia)* and "Sicilian" Lemon (*Citrus limon*) seed oils.

Parameters	"Sicilian" Lemon (*Citrus limon*)		Rangpur Lime (*Citrus limonia)*	
	^{1}H NMR*	experimental	^{1}H NMR*	experimental
Triacylglycerol average molecular weight	867.40	-	866.50	-
Saponification index	193.89	190.50	194.10	188.40
Free acidity	0.43	0.37	0.38	0.30
Iodine values	107.83	101.50	105.74	101.00
Saturated fatty acid (%)	29.30	27.00	33.20	28.20
Degree of unsaturation (%)	70.70	73.00	66.80	71.80
Linolenic acid	10.70	10.00	7.70	7.60
Linoleic acid	30.80	34.40	38.10	43.00
Oleic acid	29.20	28.60	21.00	21.20
Ratio of olefinic to aliphatic hydrogens	0.6901	-	0.7141	-

(*) values calculated from data of ^{1}H NMR integrated spectra.

The parameters determined are in good agreement with the experimental data. The R_{oa} parameter clearly indicated the oxidation state of the oils. It is more precise than the peroxide index. R_{oa} values ≥ 0.66 indicated a large degree of unsaturation and that the vegetable oil can be considered as appropriate to human consumption.

Conclusions

Our data suggest that a single analysis of ^{1}H NMR allows the determination of a large number of parameters of oil samples. ^{1}H NMR spectroscopy is very useful in food analysis, making it possible for samples to be studied in a very short period of time, providing a great deal of information and yielding similar or superior results to those obtained by classical procedures.

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