Article

Comparative Study of the Lipid Profiles of Oils from Kernels of Peanut, Babassu, Coconut, Castor and Grape by GC-FID and Raman Spectroscopy

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The characterization of the lipid profiles of oils extracted from the kernel of seeds and nuts of peanut (*Arachis hypogaea*), babassu (*Attalea speciosa*), coconut (*Cocos nucifera*), castor (*Ricinus communis*) and grape (*Vitis vinifera*) was performed by using Raman spectroscopy and gas chromatography with flame ionization detector (GC-FID). Chromatographic analyses showed that coconut and babassu oils are composed essentially by saturated fatty acids (SFA), whereas the others are composed mainly by unsaturated fatty acids (UFA). The comparison of commercial and homemade castor oils showed the former have a lower level of ricinoleic acid at ca. 58%. Spectroscopic analyses of three distinct castor oils showed their profiles could be differentiated by marker features ascribed to carbonyl modes which were correlated with storage time. The comparison between the Raman spectra of standard samples of fatty acids with the samples of oils allowed the assignment of the spectroscopic features and the characterization of marker bands for the degree of unsaturation.

Keywords: vibrational spectroscopy, chromatographic profile, handmade castor oil

Introduction

Vegetable oils are greasy, hydrophobic and unctuous to touch, with the most part of their composition having triglyceride nature; they are present in cellular organelles from mainly oleaginous fruits or grains. They are formed basically by saturated (SFA) and unsaturated (UFA) fatty acids, unsaponifiable matter and antioxidants, which makes them potentially attractive as sources of natural products for several applications in feeding and pharmacology.[1,2] The most common fatty acids in vegetable oils are oleic ($C_{18}H_{34}O_2$, octadecamonoenoic acid), linoleic ($C_{18}H_{32}O_2$, octadecadienoic acid), linolenic ($C_{18}H_{30}O_2$, octadecatrienoic acid), palmitic ($C_{16}H_{32}O_2$) and stearic ($C_{18}H_{36}O_2$) acids.

Peanut (*Arachis hypogaea*) oil is rich in UFA (oleic, linoleic and linolenic acids) and poor in SFA, while its kernel is the major source of vegetable protein, dietary fiber, antioxidant vitamins, minerals and phytochemicals.[3] Babassu (*Attalea speciosa*) oil presents high levels of fats used in food and industrial applications, as the production of

cosmetic, soap, detergent, glycerin, animal feed, lubricant and biofuel. It is composed by nearly 85% of SFA and 15% of UFA.[4,5] Coconut (*Cocos nucifera*) oil has SFA as principal components (over 80%). It is rich in lauric acid (levels over 40%), which is important to cosmetic and food industry due to its physical properties and oxidative resistance.[6,7] Grape (*Vitis vinifera*) seed oil presents high level of linoleic acid, higher than sunflower and soy oils, which is considered as the main characteristic for commercial interest.[8,9]

Castor (*Ricinus communis*) oil differs from the other vegetable oils because of its high amount of hydroxylated UFA, named ricinoleic acid ($C_{18}H_{34}O_3$). The hydroxyl group provides high viscosity and chemical stability for this compound.[10,11] In ancient times, the homemade production of castor oil was disseminated by Brazilian countryside families, which had low financial resources and difficulty in the access of health care, for its anti-inflammatory, antioxidant and laxative properties.[12]

Several analytical techniques can be applied in analysis and certification of vegetable oils such as liquid chromatography,[13,14] gas chromatography,[15-17] capillary

electrophoresis,[18,19] nuclear magnetic resonance,[20-22] and near-infrared spectroscopy.[23,24] However, in the last decade vibrational spectroscopies have been presented as an alternative, since the analysis is fast and usually the sample preparation is very simple.[25-29] Raman spectroscopy is an outstanding technique in the characterization of materials because allows obtaining finger print spectra with narrow and well resolved bands and water does not generate significant interference in the signal. This technique has been used in certification, authentication, search for adulteration of products, fatty acids determinations and in the analysis of the oxidation of oils.[30-33] The stearic, oleic, linoleic and linolenic acids have eighteen carbons in their chains, being differentiated by the degree of unsaturation. However, they can be separated by gas chromatography, as well as, identified through significant differences in some features by Raman spectroscopy.

In this context, this work characterizes the lipid profile of the oils extracted from the kernel of the nuts of peanut, coconut and babassu and the seeds of castor and grape through Raman spectroscopy and gas chromatography with flame ionization detection (GC-FID). A comparative study among three distinct castor oil samples through both techniques was also performed.

Raman spectroscopy allows inferring the chemical properties of the samples, such as the level of unsaturation and oxidation of the oils, without pre-treatment of the samples. The characterization of the lipid profile of the vegetable oils by GC-FID had the objective of obtaining quantitative information about their composition for comparing with the spectroscopic analysis. In addition, the results here obtained with both techniques and their presented interpretations have potential to be useful in applications for investigations of possible fraud, adulteration and deterioration of these oils.

Experimental

Samples

The oils extracted from the kernel of seeds and nuts of peanut, babassu, coconut, castor and grape were acquired in local market. Three distinct samples of castor oil were investigated. Besides the commercial one, two homemade was acquired, with a gap in their dates of manufacture of fifteen-year.

In the process of production of castor oil, commercial sample is extracted by cold pressing process of the seeds, whereas the homemade oil is obtained by heating process as follows: seeds are dried, powdered and heated at ca. 100-130 °C in a metallic recipient on a wood-burning stove. Water is added under stirring until the maximum of the extraction of the oil, after ca. 12 h. In the sequence equal part of water is added and the mixture is allowed to settle overnight. The organic phase is formed (extracted oil) in the presence of aqueous phase, where residual solids in suspension are trapped. The separation of the phases is achieved by simple filtration.

Reagents and materials

Standard fatty acids C14:0 (myristic acid), C16:0 (palmitic acid), C18:0 (stearic acid), C18:1c (oleic acid), C18:2cc (linoleic acid), C18:3ccc (alpha-linolenic acid), as well individual standards of fatty acid methyl esters (FAME): C16:0 (palmitic acid), C18:0 (stearic acid), C18:1c (oleic acid), C18:2cc (linoleic acid), C18:3ccc (alpha-linolenic acid) and ricinoleic acid, and a mixture of commercial standards composed by 37 fatty acid methyl esters (FAME 37), were purchased from Sigma-Aldrich, St. Louis, MO, USA. Sodium hydroxide, methanol, acetic acid, anhydrous sodium sulfate and hexane were purchased from Vetec, Duque de Caxias, RJ, Brazil. Deionized water was obtained by a reverse osmosis system.

The methanolic solution of NaOH 0.5 mol L^{-1} was prepared and stored in a plastic flask at room temperature. Stock solutions of the individual standards of FAME, at the concentration of 20.0 mmol L^{-1}, were prepared in hexane. Such solutions were stored in glass flasks in a freezer at –20 °C until the moment of analysis.

Sample analysis by GC-FID

Samples of peanut, babassu, coconut, castor (homemade and commercial) and grape oils were prepared by base catalyzed transesterification, in duplicate, as follow: 20 μL of sample were transferred to a glass tube, 3.0 mL of 0.5 mol L^{-1} NaOH methanolic solution were added and the mixture was heated in a bath at 50 °C for 10 min in a capped tube. After that, 150 μL of glacial acetic acid and 5.0 mL of 0.8% m/m NaCl aqueous solution were added and the mixture were stirred by vortexing for 15 s. Next, 3.0 mL of hexane were added in order to extract FAME's and the mixture was stirred by vortexing for 15 s. After phase separation, the upper organic one was transferred to a glass tube and the process was performed twice. The supernatant was dried by using 0.5 g of anhydrous Na$_2$SO$_4$.[34,35] The samples were stored in glass flasks at –20 °C until the moment of analysis, when they were transferred to a vial and injected into GC-FID analyzer without previous dilution.

Chromatographic analyses were performed in a GC 2010-Plus (Shimadzu, Kyoto, Japan) equipped with

a split-splitless injector, AOC 20-I auto-injector flame ionization detection. Data acquisition and analysis were performed in GC Solution software (Shimadzu, Kyoto, Japan). The column used was a CP-SIL 88 with 100 m length, internal diameter of 0.025 mm and film thickness of 0.2 μm (Agilent Technologies, Netherlands). The analysis conditions were: injector at 250 °C, flow controlled by linear speed of 26.0 cm s⁻¹, pressure at 140.3 kPa, total flow of 22.6 mL min⁻¹, injection of 1.0 μL in the split mode, initial temperature of column at 80 °C with heat ratio of 4 °C min⁻¹ up to 220 °C, isotherm at 220 °C for 5 min, heat ratio of 4 °C min⁻¹ up to 240 °C and isotherm at 240 °C for 10 min.

The analysis condition for castor oil samples were: injector at 250 °C, flow controlled by linear speed of 26.0 cm s⁻¹, pressure at 140.3 kPa, total flow of 22.6 mL min⁻¹, injection of 1.0 μL in the split mode, initial temperature of column at 80 °C with heat ratio of 4 °C min⁻¹ up to 220 °C, isotherm at 220 °C for 5 min, heat ratio of 4 °C min⁻¹ up to 240 °C and isotherm at 240 °C for 10 min, heat ratio of 3 °C min⁻¹ up to 270 °C and isotherm 270 °C for 6 min.

The normalized areas were used in the analysis of fatty acids by GC, as the recommendation of The American Oil Chemists' Society.[36,37] Hence, the areas of all peaks were integrated in the chromatogram of each fatty acid and the quantification in the oil samples was done by the ratio of the integrated areas from the respective standards. The compounds were identified by standard co-injection and retention time relative to the FAME 37 standard, a mix with 37 methyl ester fatty acids. The fatty acid concentrations were determined and expressed in g *per* 100 g of fat.

Sample analysis by Raman spectroscopy

Oil samples and fatty acids standards were analyzed in a Bruker RFS 100 instrument equipped with a Nd³⁺/YAG laser operating with wavelength at 1064 nm, and with a Ge detector cooled with liquid nitrogen. The samples, without pre-treatment, were placed in borosilicate glass tubes, which were fixed on a support and aligned with the laser beam and the light was collected at a 180° geometry. The spectra were obtained with 256 scans, using a laser power of 100 mW and spectral resolution of 4 cm⁻¹, in the range from 3500 to 200 cm⁻¹.

Results and Discussion

The separation, identification and quantification of the levels of fatty acids in the oils of peanut, babassu, coconut, grape and castor were performed through GC-FID analyses. These results were obtained based on the previous analysis of the FAME 37 mixture that was injected and used to obtain the retention time of the fatty acids of interest presented in the vegetable oil samples. The final concentrations of the fatty acids for each oil samples are presented in Table 1.

In the analysis of chromatographic profile of peanut oil sample, the predominance of palmitic, stearic, oleic and linoleic acids were verified; with UFA composition at 80.0%. In babassu oil the major fatty acids found were caprilic, capric, lauric, myristic, palmitic, stearic, oleic and linoleic acids, which gives 74.0% of SFA composition.

Table 1. Fatty acid composition in the vegetable oil samples at mass percentage

Fatty acid	Molecular formula	t_r / min	Fatty acid[a,b] / %						
			Peanut	Babassu	Coconut	Grape	CC	NCA	OCA
Caprylic	$C_8H_{16}O_2$	14	–	3.0	5.9	–	–	–	–
Capric	$C_{10}H_{20}O_2$	18	–	2.8	5.3	–	–	–	–
Lauric	$C_{12}H_{24}O_2$	23	–	41.1	49.1	–	–	–	–
Myristic	$C_{14}H_{28}O_2$	27	–	14.9	18.3	–	–	–	–
Palmitic	$C_{16}H_{32}O_2$	30	7.2	9.3	8.0	6.4	4.0	3.9	3.5
Stearic	$C_{18}H_{36}O_2$	34	2.6	2.9	3.1	3.1	2.2	2.9	2.8
Oleic	$C_{18}H_{34}O_2$	35	63.4	18.5	5.2	31.0	11.5	10.6	9.3
Linoleic	$C_{18}H_{32}O_2$	36	16.6	3.9	1.15	56.8	18.2	11.2	13.8
Eicosenoic	$C_{20}H_{40}O_2$	38	–	–	–	–	1.6	1.1	1.4
Ricinoleic	$C_{18}H_{34}O_3$	53	–	–	–	–	58.4	66.1	62.0
SFA	–	–	9.8	74.0	90.0	9.4	7.8	7.9	7.7
UFA	–	–	80.0	22.4	6.3	87.8	88.1	88.0	85.1

[a]g of fatty acid *per* 100 g of total fatty acid; [b]all measurements were performed in duplicate and relative standard deviation (%RSD) of all samples were below 5%. t_r: retention time; CC: commercial castor oil; NCA: newer homemade castor oil; OCA: older homemade castor oil; SFA: saturated fatty acids; UFA: unsaturated fatty acids.

In coconut oil the same major fatty acids from babassu oil were found, with 90.0% of SFA. In grape oil sample, palmitic, stearic, oleic and linoleic acids were identified and UFA composition was 87.8%.

In the commercial castor oil were predominant palmitic, stearic, oleic, linoleic, eicosenoic and ricinoleic fatty acids, with UFA composition at 88.1%, from which 58.4% were ricinoleic acid. From both newer and older homemade castor oils the same major components were found, with UFA composition at 88.0 and 85.1%, from which 66.1 and 62.0% were ricinoleic acid, respectively. It is noteworthy that even though the alpha-linolenic acid was chosen in detriment of its other isomers, this UFA species was not identified in all analyzed oil samples. For instance, the presence of gamma-linolenic acid is not common in vegetable oils, but it could be detected and differentiated from its isomers by GC technique.

The Raman spectra of standard samples of alpha-linolenic, linoleic, oleic, palmitic and stearic acids are presented in Figure 1. The tentative assignments of the bands, based on pertinent literature,[38-41] are presented in Table 2.

These results show that there are two distinct spectral patterns ascribed to UFA (alpha-linolenic, linoleic and oleic acids) and SFA (palmitic and stearic acids). The common bands for all these standard samples were observed with

Table 2. Tentative Raman assignment of the bands of standard fatty acids

Wavenumber / cm^{-1}					Assignment
Oleic	Linoleic	α-Linolenic	Palmitic	Stearic	
3008	3012	3014			ν_{as}(CH)
			2965	2965	ν_{as}(CH$_3$)
2933	2933	2933	2929	2929	ν_{as}(CH$_2$)
2896	2898	2902			ν_s(CH$_3$)
			2881	2883	ν_{as}(CH$_2$)
2854	2854	2856	2846	2846	ν_s(CH$_2$)
1656	1658	1658			ν(C=C)
			1465	1463	δ(CH$_2$, CH$_3$)
1440	1440	1440	1438	1438	δ(CH$_2$, CH$_3$)
			1423	1423	ν(COO$^-$)
1303	1303	1305	1297	1297	τ(CH$_2$)
1266	1265	1266			δ(CH)
			1130	1130	ν(C–C)
1077	1077	1077	1064	1064	ν(C–C)

ν: stretching, ν_{as}: anti-symmetric, ν_s: symmetric; δ: bending; τ: torsion.

different relative intensities, at 2933-2929, 1440-1438 and 1305-1297 cm^{-1}, and assigned to the asymmetric stretching of CH$_2$, bending of CH$_2$/CH$_3$ groups and torsion of CH$_2$, respectively.
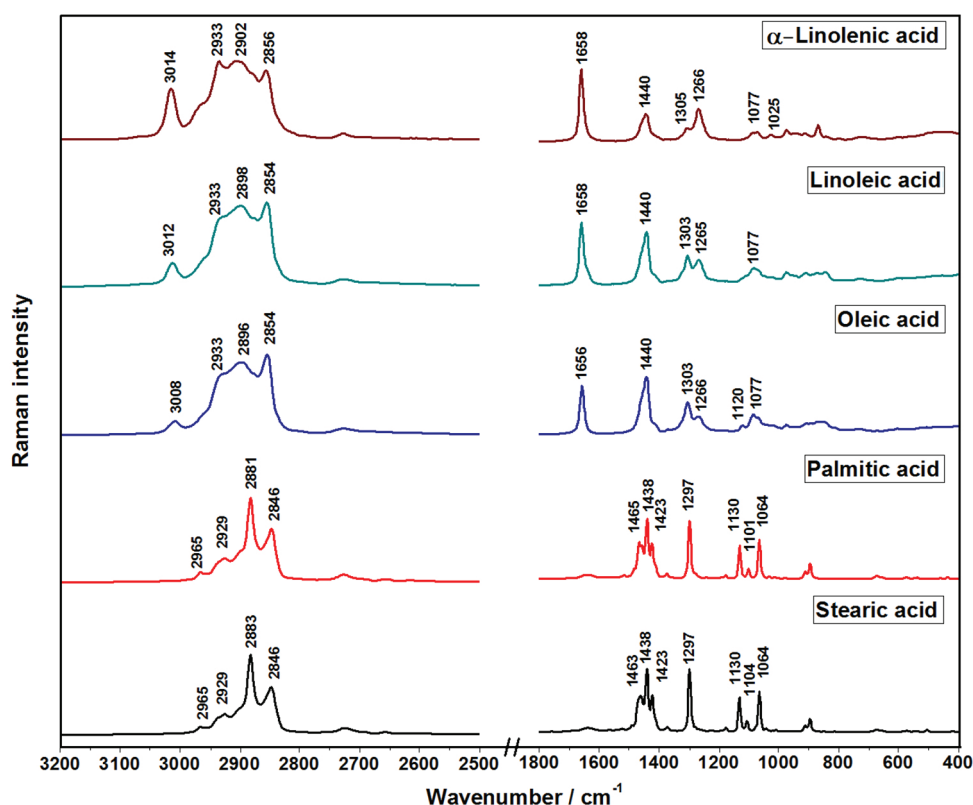

Figure 1. Raman spectra of standard samples of fatty acids. All spectra were normalized based on the intensities of the bands at 2906-2880 cm^{-1} region.

The bands at 3014-3008, 1658-1656 and 1266-1265 cm^{-1}, assigned to the stretching of CH, the stretching of C=C and the bending of CH, respectively, all involving the unsaturation moieties of UFA *cis* isomers, showed intensities proportional to the number of double bonds present in the molecular structure of the standard fatty acids. Hence, in the Raman spectra of oleic acid such bands presented one-third of the respective intensities observed in the spectra of alpha-linolenic acid and one half of those present in the spectra of linoleic acid.

Such a differentiation allows conceiving a future work in which could be used chemometrics tools, as for instance multivariate calibration, for identification of oil samples by using Raman spectroscopy.

In the Raman spectra of UFA the enlargement of the bands and the shifts to higher wavenumbers, when compared with SFA ones, are marker features for the presence of double bonds. In particular, the enlargement one can be ascribed to the breaking of the spectral degeneracy by the nonequivalence of the carbons in the polymer chains.[40]

The Raman spectra of the vegetable oils are shown in Figures 2 and 3. The assignments of the features were performed based on the Raman spectra of the standard fatty acids and the data are presented in Table 3.

It is possible to notice that the band at 1658-1656 cm^{-1}, assigned to the stretching of C=C group, presented relative intensity in accordance with the level of unsaturation, which was from 80.0 to 88.1% of UFA for peanut, grape and castor oils. Such features are ascribed to *cis* isomer of UFA and the absence of bands at 1670 cm^{-1}, assigned to the same mode from *trans* isomer, allows inferring the contamination with *trans* groups was not present in all analyzed oil samples.[38] In the spectra of babassu and coconut oils the bands at 3010 and 1656 cm^{-1}, assigned to asymmetric stretching of =C–H and stretching of C=C, respectively, presented low relative intensity, since their compositions are 74.0 and 90.0% of SFA. Therefore, the results from the assessment of the Raman spectra are in agreement with those obtained by GC-FID (Table 1). The band at 1747-1739 cm^{-1} was assigned to the stretching of C=O from triacylglycerol structure that was present in all Raman spectra of oil samples.

Commercial and homemade castor oils presented similar spectral profiles, with the Raman spectra presenting a pattern of the features characteristic of high level of UFA. However, commercial castor oil was extracted by a cold process while the homemade oils were extracted by a hot process. The similarity in the Raman spectra allows inferring the extraction methods did not interfere significantly in the composition of each castor oil sample. Nevertheless, in the older homemade castor oil, a new band at 1710 cm^{-1} was observed, which was assigned to the carboxyl moiety from the oxidation of the sample (Figure 4) that can be explained
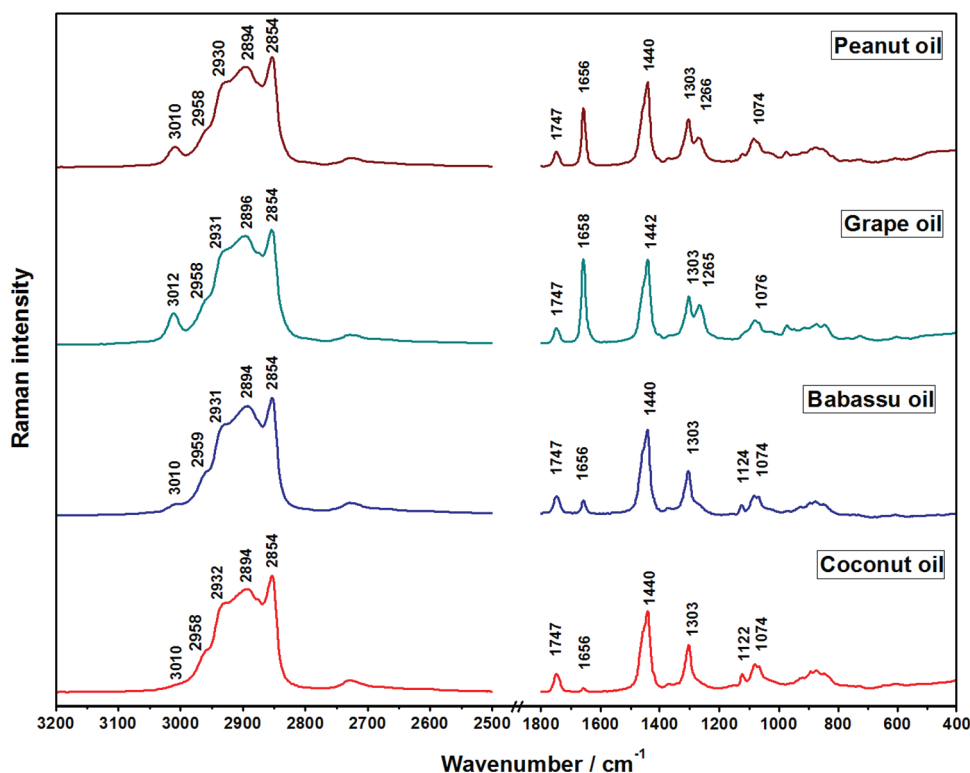

Figure 2. Raman spectra of the oils from peanut, grape, babassu and coconut. All spectra were normalized based on the intensities of the bands at 2906-2880 cm^{-1} region.
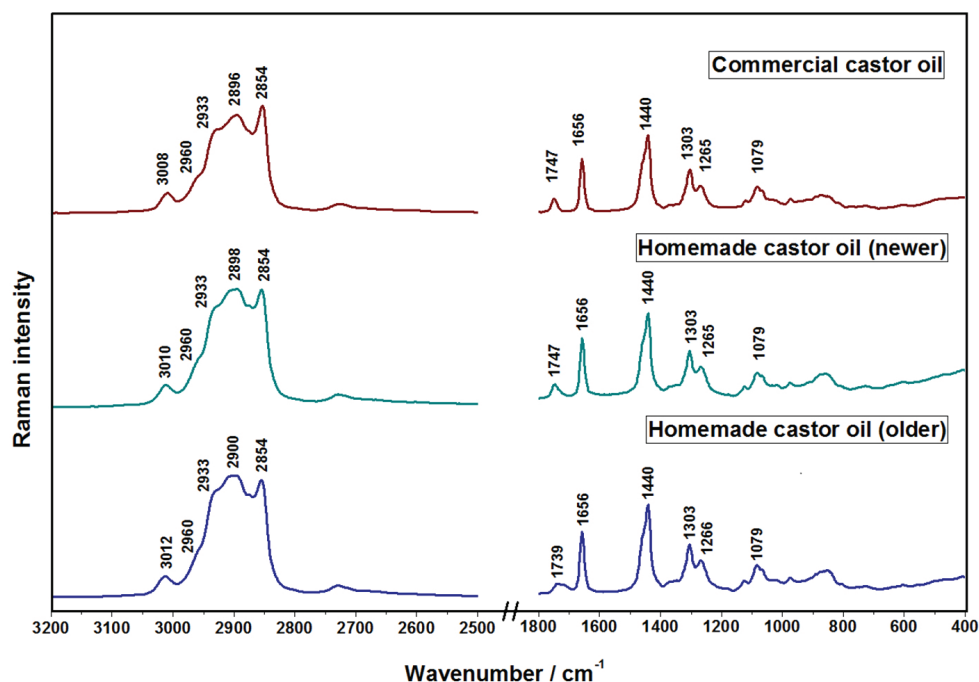
Figure 3. Raman spectra of homemade (newer and older) and commercial castor oils. All spectra were normalized based on the intensities of the bands at 2906-2880 cm^{-1} region.

Table 3. Tentative assignment of Raman bands of vegetable oils

Wavenumber / cm^{-1}							Tentative assignment
Peanut	Grape	Babassu	Coconut	CC	NCA	OCA	
3010	3012	3010	3010	3008	3010	3012	$\nu_{as}(CH)$
2930	2931	2931	2932	2933	2933	2933	$\nu_{as}(CH_2)$
2894	2896	2894	2894	2896	2898	2900	$\nu_s(CH_3)$
2854	2854	2854	2854	2854	2854	2854	$\nu_s(CH_2)$
1747	1747	1747	1747	1747	1747	1739	$\nu(C=O)$
1656	1658	1656	1656	1656	1656	1656	$\nu(C=C)$
1440	1442	1440	1440	1440	1440	1440	$\delta(CH_2, CH_3)$
1303	1303	1303	1303	1303	1303	1303	$\tau(CH_2)$
1266	1265	–	–	1265	1265	1266	$\delta(CH)$
1074	1076	1074	1074	1079	1079	1079	$\nu(C–C)$

CC: commercial castor oil; NCA: newer homemade castor oil; OCA: older homemade castor oil; ν: stretching, ν_{as}: anti-symmetric, ν_s: symmetric; δ: bending; τ: torsion.

by its long time of storage. Such a marker Raman feature from the oxidative process was not observed for all other recent produced oil samples. Chromatographic profiles for the three castor oils were quite similar, but signs of impurity in the older homemade castor oil sample were observed. In conclusion, the Raman spectroscopy showed adequate for fast evaluation of the presence of oxidized chains in vegetable oil samples, even when the presence of this species is in very low concentration.

Conclusions

The GC-FID analyses allow determining the profile

of FA in the oil samples. Some peaks presented area percentage below 1% and were not identified, but major FA quantification in the samples were not compromised. Raman spectra of the different FA and vegetable oils presented characteristic marker bands of their chemical structure and composition. The intensities of the bands at 3014-3008, 1658-1656 and 1266-1265 cm^{-1} showed correlation with the levels of unsaturated fatty acids present in the oil.

Commercial and newer homemade castor oils presented similar spectral profile; even though the commercial oil is extracted by a cold process while the homemade oil is extracted by a hot process, leading to conclude the
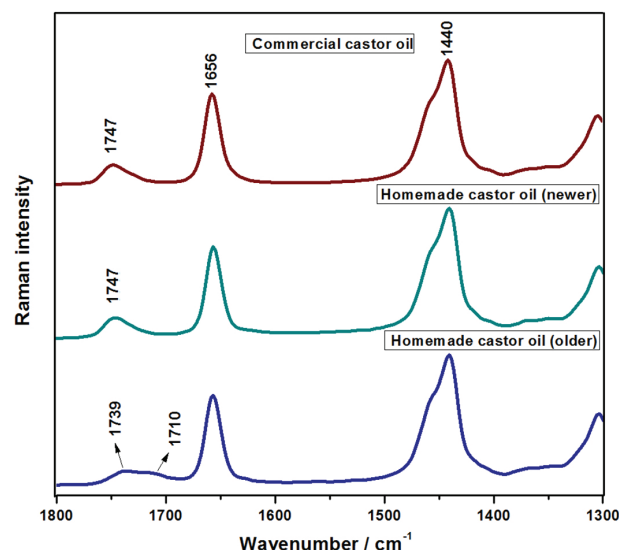
Figure 4. Raman spectra of homemade (newer and older) and commercial castor oils in the spectral region of carboxyl features.

extraction method does not interfere significantly in the oils composition. In the older homemade castor oil, a band at 1710 cm^{-1}, assigned to carbonyl moiety from the oxidation of the sample was observed, which can be rationalized by its long time of storage. However, such a marker Raman feature was not observed in all recent produced samples. Chromatographic profiles for the three samples were quite similar, but signs of impurity in the older homemade castor oil samples were observed. Raman spectroscopy revealed itself as an efficient and fast technique that can be used in the evaluation of the integrity of the oils and their esters, as well as in the search of information related to oils compositions and their degradation processes.

Acknowledgments

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