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Ultrasound assisted by microwave during the extraction of avocado oil: quality assessment by chromatographic techniques, Raman spectroscopy, and thermogravimetric analysis

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The aim of this study was to characterize the bioactive components in avocado oil (AVO) extracted by ultrasound combined with microwave, which are clean technologies used to assess oil quality. AVO samples were incubated for 30 min at 120 °C (T1), 180 °C (T2), and without any heat treatment (T3). Components were identified using chromatographic techniques, Raman spectroscopy, and thermogravimetric analysis (TGA). γ-Tocopherol and total carotenoids were affected in T2. The Raman spectrum of T3 and T1 presented a strong band at 1265 cm^{-1} related to the high content of linoleic acid and three bands associated with carotenoids. In the T2 sample, a weak intensity of the linolenic and linoleic acids was observed. In TGA, T2 showed a robust mass at 173.89 °C, characteristic of oxidized oil compounds. AVO treated at 120 °C for 30 min maintained the integrity of the bioactive compounds.

Keywords: avocado, oil, bioactive compounds, ultrasound, microwave.

INTRODUCTION

Avocado oil (AVO) has generated growing interest among consumers due to its taste, flavor, and nutritional and technological characteristics. AVO is considered a source of appreciable healthy compounds, such as fatty acids, phenolic compounds, and phytosterols[1]. AVO contains a mixture of oleic acid, palmitoleic acid, linoleic acid, linolenic acid, and saturated fatty acids, mainly in the form of triglycerides. Additionally, AVO contains other important compounds such as ß-sitosterol, campesterol, stigmasterol, avenasterol, and β-carotenoids. The percentage of compounds in AVO depends on certain factors, including maturation state, soil, and region, among others. There are different methods of extracting vegetable oils that are desirable for obtaining a good extraction yield, using both minimum amounts of solvents and short processing times. The extraction process must preserve the quality attributes of the oil, such as color, smell, aroma, and nutritional value. Some methods are cold pressing, ultrasound, microwave, supercritical CO_2, subcritical CO_2, and using organic solvents or enzymes[2]. These methods may have some advantages and disadvantages. For example, the cold extraction process produces less oil yield but a high content of α-tocopherol, squalene, campesterol, and cycloartenol acetate. On the contrary, while the use of high temperature and organic solvents during oil extraction produces high oil extraction yield, it can, however, cause oxidation of polyunsaturated fatty acids and, consequently, is known to produce rancid-tasting oil and destroy some of the bioactive compounds[3].

Microwave and ultrasound-assisted oil extraction have gained popularity because they provide good yields in a short time, use a minimum of solvents, and are clean technologies. Both extraction methods share a general principle based on using the water present in the plant cells to excite the molecules, increasing the internal temperature and pressure until the cells explode and release the oil. Microwave ovens use radio waves to transmit energy and convert it into heat at a frequency of 300 MHz to 300 GHz[4]. Dielectric energy is a form of electromagnetic energy and is the principle of microwave heating. The energy is transmitted in the form of waves by ionic conduction, penetrating into the interior of the solid, food, tissue, or cell, where it is absorbed and converted into heat. This method of microwave extraction uses the water present inside the cells by exciting the molecules, causing friction, increasing both the temperature and the internal pressure until the cells explode, releasing the compounds that can then be separated from the medium[5, 6]. The extraction of compounds by ultrasound in plant tissues is based on the propagation of ultrasonic pressure waves, where cavitation forces are generated, causing the formation of bubbles that increase in volume until they explosively collapse, releasing pressure and increasing temperature, causing the rupture of plant tissues and release of intracellular substances (e.g., oils or bioactive materials of interest) into the solvent[7]. The ultrasonic waves generally used are between 20 MHz and 100 MHz[5, 8]. A study comparing the AVO yield during the extraction using a microwave and solvents reported higher oil yield with microwaves and suggested that more steps are required for the removal of residual moisture in AVO[9]. Another study reported a high AVO yield using ultrasound and found

that peroxide and free fatty acid values were lower than the industrial specification levels, as well as an increase in total phenolic compounds[10]. The β-carotenes could be considered a control parameter to identify the loss of quality in AVO, as they are susceptible to isomerization and oxidation[11]. It was reported that microwave AVO extraction from unpeeled Hass avocado had high-quality oil with high antioxidant activity and high amounts of α-tocopherol, β-carotene, and phenolic compounds[12].

Each chemical compound in edible oil has a unique fingerprint or characteristic peaks related to its vibrational behavior in a spectral region. This region can be used to characterize compounds, such as lipids, carbohydrates, proteins, and nucleic acids, and serve to identify structures and impurities in oils. Raman spectroscopy uses scattered light to obtain information about molecular vibrational mode frequencies, which serve as the fingerprint and are useful for the identification of lipids, proteins, carbohydrates, microorganisms, and contaminants, among others, in a sample. The thermal stability of edible oils depends on the composition of fatty acids and the presence of natural antioxidants, which means that each oil has its vibrational spectrum depending on the content of distinct fatty acid molecules[13].

Several studies have been conducted to preserve the properties and nutritional value of AVO under frying conditions, such as time and temperature. In the literature, there is little information on the stability of AVO when different temperature treatments are applied. Thermogravimetric analysis (TGA) is considered a sensitive, accurate, rapid, and relatively inexpensive test that does not require sample preparation when measuring oil thermal properties. TGA consists of an oxygen absorption method used to determine the oxidative stability and some adulterant compounds in edibles oils through the measurement of weight loss during the rate heating process established in the range of 25 °C to 600 °C[14]. Also, TGA is useful to measure the stability of edibles oils such as fats, triacylglycerides, unsaturated, saturated oils that are easily susceptible to auto-oxidation process, which makes the method a valuable technique to evaluate the oxidative stability difference between oils from different sources. During the thermal oxidative decomposition processes, several consecutive and simultaneous steps of mass loss occurred in the temperature range between 200 °C and 600 °C, the first and larger decomposition step occurred in the temperature range of 200–420 °C for all oils, the second event is in the range of 420–495 °C, where the thermal decomposition of the lipid molecules started earlier is completed, and the third step occurred in the range of 495–600 °C and it is probably due to the oxidation of the carbonaceous residue[15]. In addition, it has been documented in the literature that TGA curves show thermal oxidative decomposition and mass loss of AVO in the range of 200–600 °C[16].

Microwave and ultrasound are considered emergent technologies and have been used in several applications in the food processing industry with optimal results; however, for the extraction of AVO, both methodologies have only been used individually. Therefore, the objectives of the present study were to apply the combination of microwaves and ultrasound for the extraction of AVO, quantify the tocopherols, total carotenoids, and fatty acid contents, and detect the possible thermal degradation of bioactive compounds in AVO employing chromatographic techniques, Raman spectroscopy, and TGA.

MATERIALS AND METHODS

Unripe avocado fruits (*Persea americana Mill.*cv, Haas) were purchased at a market in Morelia, Michoacan, Mexico.

Ultrasonic and microwave treatment of avocado pulp and oil separation by centrifugation

The avocado fruits were ripened at room temperature (20 °C) until visible changes in peel color (from bright green to purplish) and pulp texture (when fruits yielded slightly to finger pressure, indicating pulp softening) occurred. Then, the avocados were sanitized with 100-ppm chlorine solution. The avocados were cut in halves, and the pulp was separated from the pits and peels; then, the pulp was ground in a food processor (Black & Decker, Mod. MX 900, China). A sample of 150 g of fresh pulp was submitted under ultrasound pulse equipment (Sonics vibra cell, model VC505, Newton, CT, USA) for 2 min at 35% amplitude and 55 frequencies. Then, the pulp was placed into trays and heated by microwave (Hamilton Beach, model HB-P100N30AL-TIS) for 2 min until it reached 35 °C. Then, the dried avocado pulp was homogenized and centrifuged (CRM, Globe Centrificient IV) at 4500 rpm for 15 min until the pulp was sedimented, and the oil was filtered and collected into a dark bottle and stored at –20 °C. Three samples were prepared, of which T1 and T2 were heated for 30 min at 120 °C and 180 °C, respectively. The untreated fresh sample was used as the control (T3).

Analysis of AVO by chromatographic techniques, TGA, and Raman spectroscopy

Chromatographic techniques

Tocopherols

The content of tocopherols (α-, γ-, and δ-isomers) was determined by liquid chromatography (HPLC). AVO samples were diluted 10 times with 2-propanol, filtered through 0.45 μm nylon syringe filters, and injected (10 μL) into the HPLC system (1260 Infinity, Agilent Technologies Inc., Santa Clara, CA, USA). The HPLC system had a quaternary pump unit, a diode array detector, a fluorescence detector, an automatic sample injector, and a column oven. The separation of tocopherols was performed at 40 °C in a Discovery C18 Column (5 μm particle size, L × I.D: 250 × 4.6 mm; Supelco, Bellefonte, PA, USA) using acetonitrile/methanol (60/40, v/v) as mobile phase at a flow rate of 0.8 mL/min. Tocopherols were detected using a fluorescence detector set at an emission wavelength of 335 nm with an excitation at 292 nm. Tocopherols were identified by comparing their retention times with those of corresponding standards and by spiking samples with appropriate standards.

Fatty acid analysis

The relative composition of the fatty acids in the AVOs was determined by gas chromatography (GC) using previous preparations of the fatty acid methyl

ester (FAME) derivatives. These FAMEs were prepared by trans-esterification with a methanolic solution of potassium hydroxide[17]. Chromatographic analyses were performed using an Agilent 7890A GC system (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector, split/splitless injector, and autosampler. The GC was fitted with a HP-88 column, 100 m × 0.250 mm × 0.20 μm (Agilent). The injector port and detector temperatures were 260 °C and 280 °C, respectively. Helium at a constant flow rate of 1.0 mL/min was used as carrier gas; the split ratio was 10:1, and the injection volume was 1 μL. The initial oven temperature was 100 °C, where it was held for 4 min, then increased to 150 °C at 3 °C/min, then to 240 °C at 4 °C/min, and held for 15 min. The identification of FAMEs in AVO samples was carried out by comparing their chromatographic behavior with those of a commercial standard FAME mixture (Supelco® 37-component FAME mix).

Analysis of carotenoids

To extract carotenoids, 0.5 g of AVO samples were saponified with 3 mL of 15% KOH in methanol for 30 min at 35 °C under gently stirring. The saponification reaction was stopped by adding 9 mL of 2 M sodium phosphate buffer (pH 2.0) to the mixture. After, 10 mL hexane/dichloromethane (8:2, v/v) with 0.1% butylated hydroxytoluene (BHT) was added to extract the carotenoids, then centrifuged at 3500 × g for 2 min. The top layer was washed with 6 mL of 0.1 M sodium phosphate buffer (pH 7.0) and evaporated to dryness in a rotatory evaporator at 35 °C. Dry saponified samples were dissolved in 1 mL of methanol/methyl tertbutyl ether (1:1, v/v) with 0.1% BHT. The carotenoids were separated in a Discovery® C18 Column (5 μm, L × I.D: 250×4.6 mm) at 20 °C using the Agilent HPLC system described above. A sample of 10 μL was injected into the HPLC system via the autosampler. The elution was performed at 0.8 mL/min with mobile phase consisting of A (acetonitrile/water/ formic acid, 50:50:1, v/v), B (acetonitrile with 0.1% formic acid) and C (ethyl acetate) according to the following gradient: an initial isocratic mixture of 40% A and 60% B hold for 4 min, followed by a linear gradient to 25% A, 75% B, and 0% C at 24 min; 0% A, 100% B, and 0% C at 40 min; 0%A, 87.5% B, and .5% C at 44 min; 0% A, 60% B, and 40% C at 46 min; 0% A, 30% B, and 70% C at 58 min; 0% A, 100% B, and 0% C at 62 min, hold for 4 min; the initial conditions were re-established in 2 min and hold for 15 min. The UV-visible absorption spectra were obtained in the 200 nm to 600 nm range, and carotenoids were monitored at 450 nm. The carotenoids were identified in oil samples by comparing their retention time and absorption spectra with the reference standards. UV-visible spectra features (maximum wavelength absorbance) and a comparison of elution order to previously published literature[18–21] were additionally used to confirm carotenoids without commercially available standards. Lutein equivalents were used to calculate violaxanthin, neoxanthin, and lutein-5,6-epoxide content. The total carotenoid was calculated as the sum of all carotenoid components identified in the AVO. Results were expressed as milligrams of total carotenoids per kilogram of AVO.

Thermogravimetric analysis (TGA)

Mass loss as a function of temperature from each sample of AVO was determined in a thermogravimetric analyzer (Pyris-1 TGA, PerkinElmer, USA). Approximately 5 mg of each sample was subjected to runs from 25 °C to 600 °C, with a heating rate of 10 °C/min and purging with nitrogen. TGA data were expressed as percent mass loss as a function of temperature.

Raman spectroscopy

All the spectra from AVO samples were acquired with a Raman spectrometer (LabRam HR Evolution, HORIBA Scientific) equipped with a laser operating at 785 nm, with an excitation power of 50 mW, and an Ar-ion laser emitting 5 mW at 488 nm. The spectral range in both cases ranged from 700 m^{-1} to 2000 cm^{-1}. The spectral resolution of this instrument is less than 1 cm^{-1}. For each scan, the data acquisition time was set to 30 seconds for 488 nm and 15 seconds for 785 nm to obtain appropriate signal-to-noise ratios. The Raman band wavenumbers have accuracy and precision below 2 cm^{-1}. The measurements were done by focusing the laser line on one drop of oil placed in a small well (~1 mm in diameter) in a metallic plate.

RESULTS AND DISCUSSION

Three samples of Hass variety AVO were investigated. The first sample was heated for 30 min at 120 °C (T1), and the second was heated for 30 min at 180 °C (T2). The control sample (T3) was the unheated oil.

Characterization of AVO using chromatographic techniques, thermogravimetric analysis, and Raman spectra analysis

Tocopherols, carotenoids, and fatty acids content in AVO by chromatography

Table 1 shows the results obtained from the analysis of tocopherols. The **α**-tocopherol content found in AVO (T3) was higher than the value reported in the literature, 60.4 mg/Kg[22], in the variety Margarida. In reference to the heat treatments in our study, **α**-tocopherol was the only affected component since it showed a tendency to degrade depending on the applied temperature, with a decrease in its concentration of 7.78% in T1 and 14.73% in T2.

Table 1. Tocopherol concentration (mg/kg oil) and total carotenoids (mg/kg oil) in AVO submitted at 120 °C and 130 °C for 30 minutes

Compound	T1	T2	T3
α	120.8 ± 0.60	111.7 ± 0.63	131.0 ± 0.63
γ	8.1 ± 0.33	7.9 ± 0.33	8.0 ± 0.08
δ	4.5 ± 0.20	4.4 ± 0.18	4.3 ± 0.05
Total tocopherols	133.4	124.0	143.3
Total carotenoids	19.94 ± 0.18	9.70 ± 0.10	20.64 ± 0.21

The concentration of γ-tocopherol and δ-tocopherol were kept constant. Table 1 shows the results of total carotenoid compounds. In general, the treatment at 180 °C (T2) drastically affected total carotenoid compounds compared to T3. The decrease in total carotenoids with respect to the control (T3) was only 3.39% in T1 but was reduced by 53.0% in T2.

The results of the fatty acids analysis by gas chromatography are shown in Table 2. In general, the treatment applied to AVO with temperatures of 120 °C and 180 °C did not alter the concentration of saturated and unsaturated fatty acids. There was a 6.19% decrease in T2 observed compared to the control. Moreover, we observed the appearance of linolelaidic acid at 5.0% in T3, possibly because the isomerization process occurred due to temperature, where linolenic acid (C18:2n6*c*) had a rearrangement in its structure, and linolelaidic acid (C18:2n6*t*) was formed.

It is important to mention that the oil remained stable before the exposure of the oils to heat treatment and the presence of light and oxygen, possibly due to the presence of tocopherols and carotenoids, and therefore, the fatty acid profile remained unchanged in T1 and T2. The presence of tocopherols inhibits autooxidation in oils by inhibiting the initiation and propagation of free radicals because they donate their phenolic hydrogen to lipid-free radicals present. Carotenoids are antioxidants that quench singlet oxygen, an intermediate in photooxidation reactions[23].

Different spectroscopic techniques were used to monitor the changes observed in tocopherols, carotenoids, and fatty acids in AVO heated at 120 °C (T1) and 180 °C (T2) and in the unheated control oil (T3).

Thermogravimetric analysis

TGA curves of T1, T2, and T3 are shown in Figure 1a. The TGA curves revealed the following thermal stability sequence: T3>T1>T2. The main thermal degradation of treatments is shown in the region of desiccation associated with the evaporation of water. For T1, this range was from 100 °C–369.26 °C, and from 100 °C–372.36 °C for T3 (Fig. 1a). Mass loss was higher in T3 (4.90%) than in T1 (4.21%). T1 (120 °C/30 min) had less water content. T3 (no thermal treatment applied) maintained all its characteristic compounds and, for this reason, had high thermal stability. This behavior in T3 was similar to that reported in the literature[23], in which it was mentioned that oil extracted via microwave was of high quality with elevated antioxidant activity due to the high presence of α-tocopherol, carotene, and phenolic compounds. In T2 (180 °C/30 min), the temperature of 348.44 °C was lower than T1 and T3, but in this case,

Table 2. Composition (%) of fatty acids analyzed by gas chromatography of AVO submitted at 120 °C and 130 °C for 30 minutes

Fatty acid	T1	T2	T3
Palmitic acid (C16:0)	17.0 ± 0.15	17.0 ± 0.12	17.4 ± 0.10
Palmitoleic acid (C16:1)	7 ± 0.10	6.9 ± 0.10	7.2 ± 0.08
Stearic acid (C18:0)	0.5 ± 0.01	0.4 ± 0.02	0.5 ± 0.01
Oleic acid (C18:1n9c)	63 ± 0.95	59.1 ± 0.72	63 ± 0.81
Linoleic acid (C18:2n6*c*)	10.6 ± 0.07	10.4 ± 0.07	10.6 ± 0.09
Linolenic acid (C18:3n3)	0.7 ± 0.01	0.7 ± 0.02	0.7 ± 0.01
Linolelaidic acid (C18:2n6*t*)	0	5.0 ± 0.18	0
cis-11-eicosenoic acid (C20:1)	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01
Others	1.0	0.3	0.4

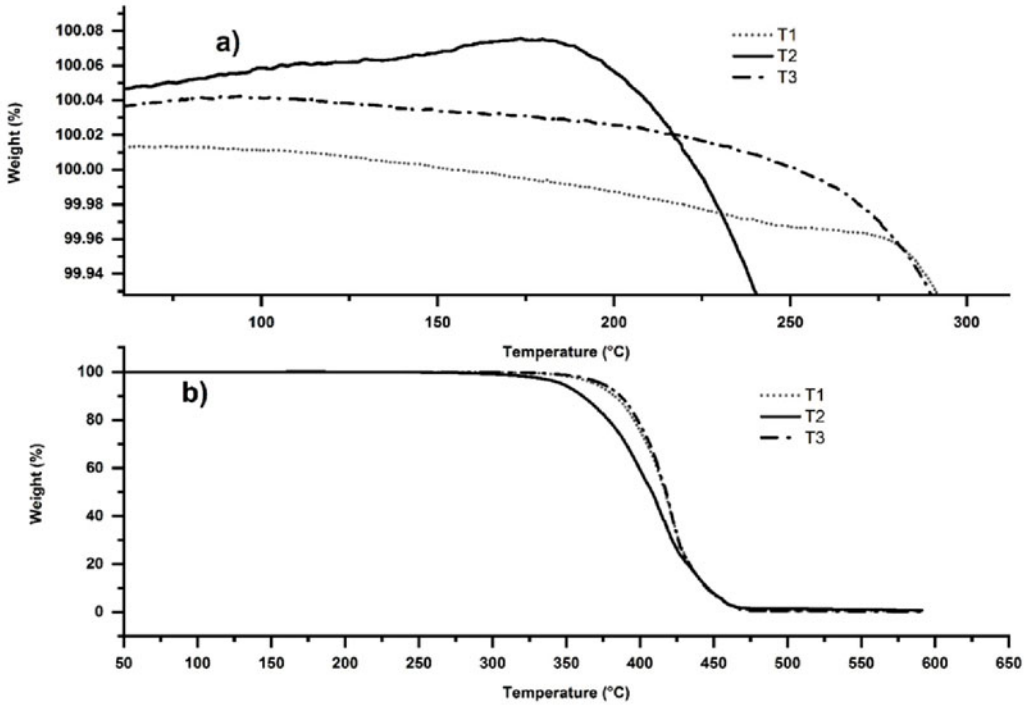

Figure 1. TGA from samples of AVO extracted with emergency technology combination and heated for 30 min at 180 °C (T2, solid line), 120 °C (T1, medium line), and control sample (T3, dotted line). a) Temperature from 0 °C to 300 °C. b) Temperature from 50 °C to 600 °C

with a mass gain of 0.01% at 173.89 °C. Some studies reported that a temperature of 180 °C could promote lipidic oxidation in oils, producing compounds such as aldehydes, ketones, and alcohols able to react among themselves or with water molecules[24]. The degradation oil pathways can be formed through C-C bond cleavage, elimination by hydrolysis, or thermal rearrangement[13, 16, 25]. Under heating, AVO is susceptible to degradation, thus changing its organoleptic properties. It was documented that polyunsaturated fatty acids were degraded in a temperature range of 370.81 °C to 475.86 °C[8]. In our study, a mass loss of 98.63%, 93.47%, and 99.46% were found in T1, T2, and T3, respectively, for the decomposition of monosaturated fatty acids at temperatures ranging from 475.86 °C to 600 °C, as can be seen in Figure 1b. T3 and T1 had lower mass loss than T2. T1 and T3 had a high amount of polyunsaturated fatty acids and antioxidant compounds.

Raman spectra

The representative vibrational modes in edible oils can be identified with Raman in the range of 800–1800 mainly, for instance, for the identification of molecules of interest in AVO, such as carotenoids (Fig. 2a) and unsaturated fatty acids, such as oleic and linoleic acids (Fig. 2b). Each molecule has a particular intramolecular vibrational level, and this can be used to evaluate the oil quality as well as its authenticity.

Raman spectroscopy was used to verify the authenticity of olive oil[26]. It is important to mention that the vicinity of the main peaks found in the olive oil was similar to those found in our study. The bands at 1265 cm^{-1} and 1657 cm^{-1} correspond to the cis-(=C-H) vibration and the cis-(C=C) vibrations, respectively, which can be correlated to the content of high-unsaturated fatty acids in the oils. In the AVO sample, the band at 1265 cm^{-1} was remarkably higher than that of olive oil, meaning that oleic and linoleic are in a higher content. These bands are important because they relate to the percentage of monounsaturated (oleic) and polyunsaturated (linoleic) fatty acids. Linoleic acid has two double bonds C=C, contributing to the strong intensity at 1657 cm^{-1}. Using a 785 nm excitation light, one of the three bands associated with carotenoids was observed at 1527 cm^{-1}. The C=C stretch is commonly used as a reference point to identify the authenticity of extra virgin olive oil due to its natural color. However, in our study, it was demonstrated that not only using the 785 nm laser but also the 488 nm excitation light is required to identify the carotenoids content in AVO at bands of 1004 cm^{-1}, 1156 cm^{-1}, and 1525 cm^{-1} (Fig. 2a), which have even stronger intensity than olive oil. In avocado pulp, the major components in the oil were oleic (C18:1), palmitic (C16:0), linoleic (C18:2), and palmitoleic (16:1) acids. The band at 1657 cm^{-1} is indicative of the degree of polyunsaturated fatty acids and a high concentration of linoleic fatty acid, which in this case is stronger than that reported in the literature about other AVO samples and the lowest for olive oil, which has a high content of monounsaturated oleic fatty acid[27, 28]. As the peak wavenumber decreases to 1306 cm^{-1}, marked for alkanes, there is an increase in the content of double bonds (unsaturation). Peaks found at 972 cm^{-1}, 1263 cm^{-1}, and 1657 cm^{-1} are related to the degree of unsaturation (the number of C=C bonds), and the peaks at 1303 cm^{-1} and 1441 cm^{-1} are related to saturated fatty acids (CH_2)[29]. The degree of the unsaturation of fatty acids can be used to identify the oil quality. The peak at 1657 cm^{-1} showed an increase as a function of the degree of oil unsaturation, which can be used as a standard related to a variation among

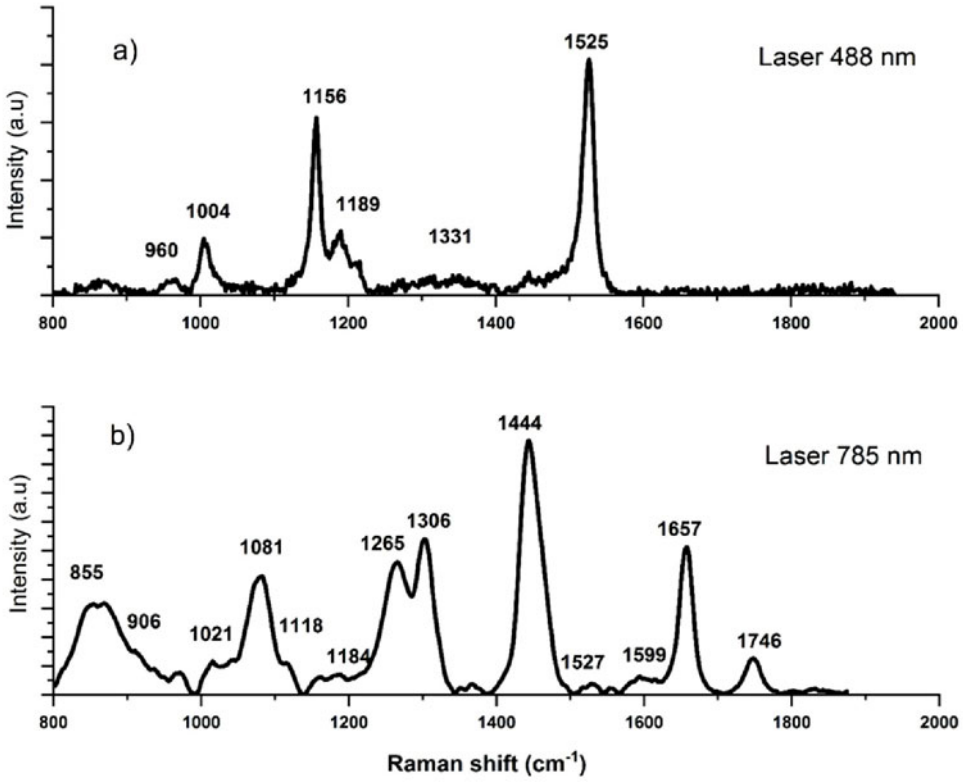

Figure 2. Raman spectra from AVO (T3) sample, applying different lasers: a) 488 nm and b) 785 nm light

commercial AVO. Moreover, in this case, our AVO has a similar tendency as olive oil but with the remarkable intensity of the 1265 cm^{-1} peak.

The variations in the relative intensity of bands in T1, T2, and T3 samples and the occurrence of other weak bands or shoulders are attributed to differences in the temperature applied to the fatty acid composition. The availability of more data for AVO via Raman spectroscopy would further facilitate the identification of adulterants and the classification of commercial oils. It would also provide a way to guarantee the mandatory labeling of quality AVO, especially if they are being used at an intensity of three bands of around 1004 cm^{-1} (C-CH_3 bend), 1156 cm^{-1} (C-C stretch), and 1525 cm^{-1} (C=C stretch), corresponding to carotenoids, shown in Figure 2a, with a 488 nm laser line. These spectral bands have been related to the quality characteristic of oils, such as olive oil[30, 31], and, according to our results, could be extended to AVO. The bands at 1265 cm^{-1} and 1657 cm^{-1} correspond to oleic and linoleic acids. The Raman test requires more study because edible oils, such as AVO, have a photoluminescent behavior, which overlaps important signals when oxidized. This was the main reason it was only presented on the Raman spectra of sample T3 since the spectra of samples T2 and T1 presented a strong photoluminescence. Table 3 shows all the frequencies of the main vibrational modes present in AVO compared to those observed in olive oil.

CONCLUSIONS

In this work, a combination of ultrasound-assisted with microwaves was used for the first time to obtain AVO. AVO showed healthy compounds, including linolenic acid (ω3), linoleic acid (ω6), tocopherols, and total carotenoids. On the other hand, the AVO suffered changes under heat (180 °C/30 min), and some healthy compounds were affected, mainly γ-tocopherol and total carotenoids. A temperature of 120 °C did not induce chemical changes in AVO. The combination of ^{1}H-NMR and Raman spectroscopy with TGA was useful for the simultaneous determination of AVO quality and was associated with changes in tocopherols and carotenoids. Finally, Raman spectroscopy represents a promising alternative technique to measure the quality of AVO.

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Table 3. Bands assignment in the Raman spectra of avocado and olive oils

Assignment	AVO		Olive oil
	Laser 785 nm	Laser 488 nm	
C = C bend (trans)	966		966w
C-CH_3 bend		1004	
C-C (CH_2) stretch	1080		1078
C-C stretch		1156	
=C-H	1265		1270s
-CH_2	1298		1306
CH_2 twist	1306		1306
O–CH_2 wag	1368		1368s
C-H- scissoring	1444		1444s
C=C stretching	1527	1525	
C=C stretching	1657		1651s
C=O stretching	1746		1738m

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