Detection of olive oil adulteration by low-field NMR relaxometry and UV-Vis spectroscopy upon mixing olive oil with various edible oils

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SUMMARY: Adulteration of olive oil using unhealthy substitutes is considered a threat for public health. Low-field (LF) proton (1H) nuclear magnetic resonance (NMR) relaxometry and ultra-violet (UV) visible spectroscopy are used to detect adulteration of olive oil. Three different olive oil with different oleoyl acyl contents were mixed with almond, castor, corn, and sesame oils with three volumetric ratios, respectively. In addition, Arbequina olive oil was mixed with canola, flax, grape seed, peanut, soybean, and sunflower seed oils with three volumetric ratios. Transverse magnetization relaxation time (T_2) curves were fitted with bi-exponential decaying functions. T_2 times of each mixture of olive oils and castor oils, and olive oils and corn oils changed systematically as a function of volumetric ratio. To detect the adulteration in the mixtures with almond and sesame oils, both LF ^{1}H NMR relaxometry and UV-Vis spectroscopy were needed, where UV-Vis-spectroscopy detected the adulteration qualitatively. In the mixtures of Arbequina olive oil and flax, peanut, soybean, and sunflower seed oils, both T_{21} and T_{22} values became longer systematically as the content of the olive oil was decreased. The unique UV-Vis maximum absorbance of flax oil at 320.0 nm shows the adulteration of olive oil qualitatively.

KEYWORDS: *Adulteration detection; Low-field NMR relaxometry; Olive oil; UV-Vis spectroscopy*

RESUMEN: *Detección de la adulteración de aceite de oliva mediante relaxometría magnética nuclear de campo bajo y espectroscopía UV-Vis sobre mezcla de aceite de oliva con diversos aceites comestibles*. La adulteración del aceite de oliva con sustituyentes menos saludables es una amenaza para la salud pública. En este trabajo, la detección de la adulteración del aceite de oliva se demuestra utilizando tanto relaxometría magnética nuclear de campo bajo (LF) de protones (^{1}H) (RMN) y espectroscopía visible y ultra-violeta (UV). Tres muestras de aceites de oliva con diferentes contenidos en oleico se mezclaron con aceites de almendra, ricino, maíz y sésamo con tres relaciones volumétricas. Además, el de arbequina de California se mezcló con cánola, lino, semilla de uva, cacahuete, soja y aceites de girasol con tres relaciones volumétricas. Las curvas de tiempo de relajación de magnetización transversal (T2) fueron completadas con funciones de decaimiento biexponencial. Los tiempos T2 determinados para cada mezcla de aceite de oliva con ricino y maíz cambian sistemáticamente conforme las relaciones volumétricas. Para detectar la adulteración de los aceites de oliva con aceites de almendra y de sésamo, se necesitan tanto relaxometría LF 1H RMN como la espectroscopía UV-Vis. En las mezclas de aceite de oliva arbequina con aceites de lino, maní, soja y girasol, ambos valores T21 y T22 se hicieron mayores sistemáticamente conforme el porcentaje de aceite de oliva se reduce en la mezcla. El máximo de absorbancia UV-Vis del aceite de lino a 320,0 nm demostrará la adulteración del aceite de oliva cualitativamente.

PALABRAS CLAVE: *Aceite de oliva; Detección de adulteración; Espectroscopia UV-Vis; Relaxometría RMN de campo bajo*

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1. INTRODUCTION

Olive oil is a "natural fruit juice" obtained from the fruit of *Olea europea* L. by mechanical or physical procedures (Zamora *et al.* 2001). The consumption of olive oil has increased recently due to its sensory qualities such as taste and health benefits, namely the reduction of risk factors and prevention of the occurrences of chronic diseases like diabetes and obesity (Rohman and Che Man, 2010; Yang and Irudayaraj, 2001; Mendes *et al.*, 2015). In addition to the increase in olive oil consumption, there are unfortunately malpractices in olive oil production including adulteration. Olive oil is usually adulterated by blending with either pomace olive oil or other cheap edible oils including corn, peanut, and sunflower oils. Such malpractice causes threats and challenges to the suppliers and the health of the ultimate consumers (Gurdeniz and Ozen, 2009; Lizhi *et al.*, 2010). For example, because of the consumption of fake cooking mustard oil, more than forty people died in 1998 in India (Lizhi *et al.*, 2010). Therefore, it is essential to develop viable techniques for detecting adulterated edible oils.

In order to detect olive oil adulteration upon blending olive oil with various oils such as hazelnut oil (Zabaras, 2010), palm oil (Rohman and Che Man, 2010), corn oil (Lizhi *et al.*, 2010), and almond oil (Dourtoglou *et al.*, 2003), different techniques ranging from Fourier Transform (FT) infrared (IR) (Rohman and Che Man, 2010; Gurdeniz and Ozen, 2009) and dielectric spectroscopy (Lizhi *et al.*, 2010) to UV-Vis spectroscopy (Torrecilla *et al.*, 2010a) have been employed. Furthermore, both high- (Smejkalova and Piccolo, 2010; Agiomyrgianaki, 2010) and low-field NMR (Xu, 2014) techniques have been utilized. Besides this, the results of the studies were analyzed by chemometrical approaches such as principal component analysis (PCA) (Gurdeniz and Ozen, 2009; Lizhi, 2010).

In the present contribution, both LF NMR relaxometry and UV-Vis spectroscopy were applied. Recently, LF NMR has been employed in food science as a preferred technique due to its advantages such as being rapid, non-destructive, highly reproducible, and sensitive (Riberio *et al.*, 2014, Hills 2006). UV-Vis spectroscopy was also applied in analyzing olive oil because UV-Vis is a less time consuming method in terms of sample preparation and data processing (Torrecilla, 2010a). It is also easy to monitor the presence of colorants of chlorophylls and the derivatives in olive oil samples by UV-Vis spectroscopy (Torrecilla *et al.*, 2010b; Roca *et al.*, 2010). Edible almond, castor, corn and sesame oils were picked for mixing with three different olive oil samples having different oleoyl acyl contents. Among the three olive oil samples, Arbequina olive oil from California was also mixed with canola, flax, grapeseed, peanut, soybean, and sunflower seed oils with three volumetric ratios. The adulterant oils of interest in the current study are cheaper than olive oil, and have similar densities to that of olive oil. Therefore, it is important to detect olive oil adulteration in particular when olive oil is mixed with such cheap and edible oils. The aim of the present research is to test olive oil adulteration detection by rapid and complementary techniques of LF NMR relaxometry and UV-Vis spectroscopy, when in particular the adulteration is at and above a critical economic level. In achieving this goal, the approach of the current contribution is to develop a general predictive strategy with a larger set of representative signals, which might be applied in common analysis laboratories.

2. MATERIALS AND METHODS

2.1. Samples

Chloroform was purchased from Sigma-Aldrich with 99.9% purity. Arbequina and Koroneiki olive oils from California were received as gifts from a Californian company with the production date of March 19, 2014, while French-Nyons olive oil was obtained from a local market in Columbus, Ohio USA, and it was the product of the harvest season in the fall of 2013. The chemical contents of these three olive oils were mentioned previously (Ok, 2016). The other oil samples were purchased from local markets in Columbus, Ohio, USA (Table 1). The geographical origins of these adulterant oils are given in Table 1. Prior to the measurements, all of the olive oil samples were kept at 20 °C in the dark under continuous air conditioning. The olive oil sample was blended for the LF NMR measurements with the other oil samples by three different volume ratios of olive oil to the corresponding oil (v/v 2:1, 1:1, and 1:2). The transverse magnetization

TABLE 1. List of the oils used for blending with olive oil samples

Sample	Property as Sold	Origin
Almond Oil	-	USA
Canola oil	-	USA and Canada
Castor Oil	-	India
Corn Oil	Pure	USA
Flax seed Oil	-	Egypt
Grape seed Oil	Pure	Italy
Peanut oil	100% pure	USA
Sesame Oil	Extra virgin	India
Soybean oil	pure	USA
Sunflower seed oil	100% pure	Turkey

relaxation time (T_2) measurements on the LF-NMR instrument made with properly sealed 10 mm NMR tubes. Mixing was carried out by ultrasonication for three minutes at 25 °C prior to the measurements. The volumetric ratio of the mixtures for UV-vis spectroscopy measurements was always 1:1.

2.2. LF^{1}H NMR relaxometry

LF T_2 NMR measurements were performed on a Bruker Minispec mq20 NF Series instrument with a magnetic field strength of 0.47 T corresponding to a proton resonance frequency of 20 MHz at a magnet temperature of 40 °C. The instrument is equipped with a 10 mm temperature-variable probe. Transversal (T_2) relaxation was measured using the standard Carr-Pucell-Meiboom-Gill (CPMG) pulse sequence found in the Bruker library. The T_2 measurements were conducted with a time delay between the 90° and 180° pulses (τ) of 1.0 ms. Data from 1250 echoes were acquired from 32 scans, and the delay time was chosen long enough, $5*T_1$, to enable complete decay of the T_2 signal (Ok, 2014). The repetition time between two succeeding scans was set to 10 s.

The NMR transverse relaxation data were analyzed as follows: a continuous distribution of T_2 exponentials related to fatty acyl contents was fitted for all CPMG using the CONTIN algorithm (Provencher, 1982). This analysis yielded a plot for the continuous T_2 distribution. Bi-exponential fitting analysis of T_2 relaxation data was performed using Origin 9.1 employing the following equation:

$$y = A_{21}*\exp(-x/T_{21}) + A_{22}*\exp(-x/T_{22}) \text{ (eq.1)}$$

Where T_{21} and T_{22} are the relaxation components, and A_{21} and A_{22} are the corresponding amplitudes. The amplitudes A_{21} and A_{22} are directly proportional to the amount of fatty acyl present in the samples. The T_{21} component was calculated as $A_{21}/(A_{21} + A_{22})$ while T_{22} component was determined as $A_{22}/(A_{21} + A_{22})$ (Aursand et al., 2008).

2.3. UV-Vis spectroscopy

The absorption spectra of the thermally treated olive oil samples were obtained in the range of 800-234 nm, at 1 nm resolution, using Shimadzu UV-2401PC UV-Vis Recording Spectrophotometer at 25 °C. Quartz cuvettes were used for obtaining the UV-Vis spectra. 0.05 ml of sample was diluted with 3 ml of $CHCl_3$ prior to the measurement.

3. RESULTS

First, the detection of olive oil adulteration was evaluated by fitting transverse magnetization relaxation time (T_2) of the blended samples (Figure 1, Table 2). Two issues were focused on: bi-exponential fitting of T_2 relaxation decaying curves and continuous distribution of the relaxation curves obtained by Inverse Laplace transformation. It was shown previously that Arbequina, Koroneiki, and France-Nyons olive oils had approximately 72%, 79%, and 84% oleoyl acyl contents, respectively (Ok, 2016). This difference in oleoyl acyl contents of the olive oils was reflected in the T_2 values. Arbequina olive oil with the lowest oleoyl acyl content had the shortest T_2 time. Figure 1 shows representative T_2 decays of Arbequina olive oil, castor oil and their mixtures with v:v/1:1. T_2 of pure olive oil reached complete decay above 1250 ms, while that of pure castor oil reached complete decay above 440 ms. The T_2 decay of the mixture of Arbequina olive oil and castor oil with v:v/1:1 showed complete decay above 1000 ms. There is also the effect of the oleoyl acyl fraction in the T_2 values of the oil mixtures. As can be seen in Table 2, the blends of almond oil and either Arbequina or Koroneiki olive oil, as well as the mixtures of sesame oil and either of the two olive oils did not show significant changes in T_{21} and T_{22} times with respect to those of pure almond and sesame oils. Changing the volumetric ratio of the two olive oils (Arbequina and Koroneiki) to either almond oil or sesame oil did not affect the ultimate T_{21} and T_{22} relaxation values. This was related to the similar densities of the almond, sesame and olive oils. In other words, the fatty acyl fractions in sesame, almond, and olive oils are similar to each other. Thus, changing the volumes in preparing these particular blends did not change the ratios of the fatty acyls in the samples. This in turn led to very close T_2 relaxation values. However, T_{21} values of the mixtures of France-Nyons olive oil and almond oil were longer than that of pure almond oil, while T_{21} values of the mixtures of France-Nyons olive oil and sesame oil were shorter than that of pure sesame oil. These examples clearly indicate a difference in the oleoyl acyl contents of olive oil samples as an important factor in following the representative signals of adulterated olive oils. As shown in Figure 2 and listed in Table 2, when olive oil samples were mixed with castor oil, both T_{21} and T_{22} values became shorter as the volumetric ratio of olive oil to castor oil was changed from 2:1 to 1:1, and even further to 1:2. This was independent of which olive oil was used for preparing the mixtures. The most interesting result was observed in the case of the mixtures prepared by mixing the olive oil samples with corn oil. Both the T_{21} and T_{22} values became longer as the volumetric ratios of the olive oils to corn oil were increased. However, the two values did not become as long as the ones belonging to the pure corn oil.

Then the adulteration detection of olive oil was analyzed by focusing on data of the mixtures of Arbequina olive oil with the other oils of canola, flax, grape seed, peanut, soybean, and sunflower seed. As listed in Table 2, T_{21} and T_{22} values of the
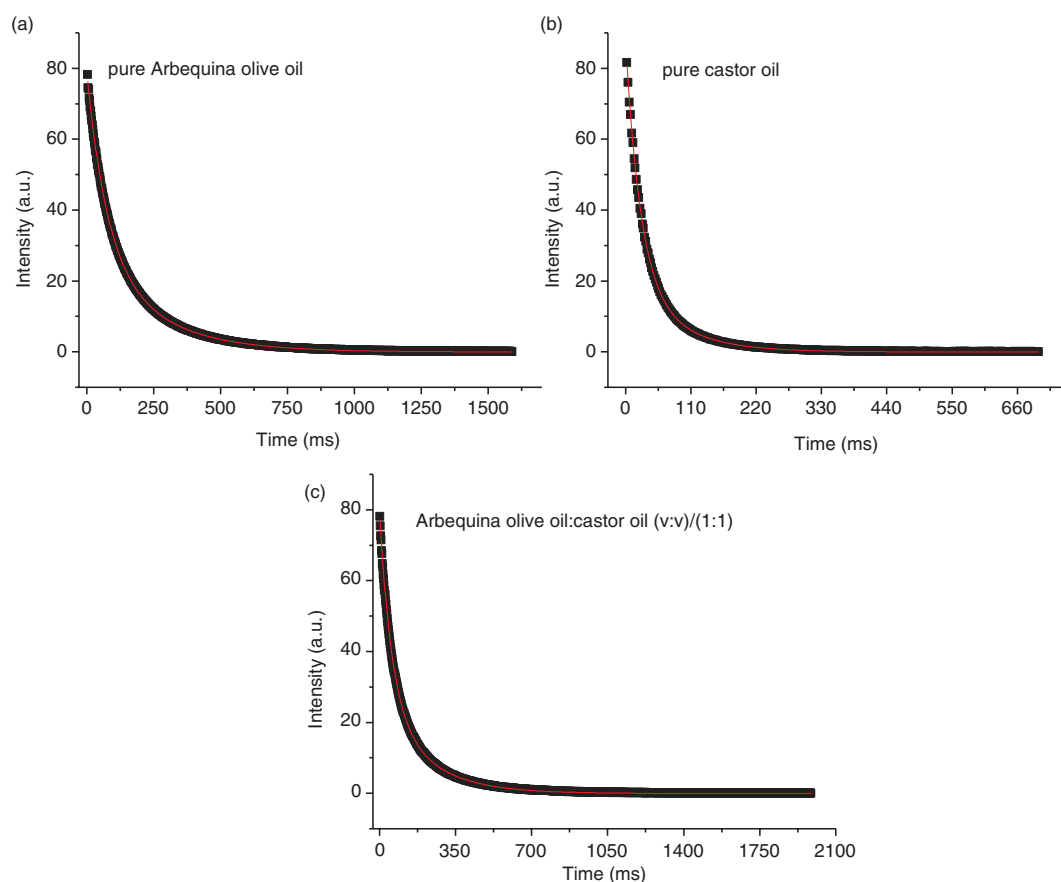
FIGURE 1. Typical examples of T_2 data bi-exponential fittings.

mixtures of Arbequina olive oil and canola oil were shorter than these of pure canola oil. T_{21} and T_{22} values did not change when the volumetric ratio of Arbequina olive oil to canola oil varied from 2:1 to 1:1, and 1:2. There were no systematic changes in T_{21} or T_{22} values of the mixtures of Arbequina olive oil and grape seed oil when the volumetric ratios were changed systematically. However, in the mixtures of Arbequina olive oil with the oils of flax, peanut, soybean, and sunflower seed, both T_{21} and T_{21} values were longer than those of pure olive oil, and shorter than those of pure oils. In these mixtures of Arbequina olive oil and flax, peanut, soybean, and sunflower seed oils, both T_{21} and T_{22} values became longer systematically as the percentage of the olive oil was decreased in the mixtures.

Figure 2 shows typical T_2 distributions obtained using the Contin software of the pure olive oil samples, castor oil, and blends of each olive oil with the castor oil. Similar results were observed in the case of the other blends (see Figures 3, 4, and 5 showing T_2 distributions of olive oil samples with almond, corn, and sesame oils). The distributed exponential approach utilizes a regularization method to the

inverse Laplace transformation yielding a continuous distribution of T_2 relaxation times (Provencher 1982). Mathematically, the distributed exponential fitting is ill-defined because it is sensitive to the constraints employed (Hansen *et al.*, 2010; Martens and Thybo, 2000). In addition, there is a shorter T_2 relaxation component observed in the range of 10–15 ms. This component is usually attributed to a processing artifact arising in the Contin processing step (Aursand *et al.*, 2008). For this reason, this T_2 relaxation component in the range of 10–15 ms is not taken into account in evaluating the T_2 distribution of the oils.

As shown in Figure 2(a), when the volume of castor oil in the blend with Arbequina olive oil was increased, the relaxation curves shifted toward the T_2 distribution curve of pure castor oil systematically. In addition, the peak of pure castor oil at 25 ms emerged with the two peaks of pure olive oil below 180 ms giving rise to a single peak at around 60 ms. The peak of pure olive oil at 256 ms also shifted to shorter values as the amount of castor oil in the blends was increased. These results showed the possibility of monitoring the adulteration of

TABLE 2. T_2 values of oils in their pure state and in blends with olive oil samples

Pure oils	A_{21}	A_{22}	T_{21} (ms)	T_{22} (ms)	% A_{21}	% A_{22}
California-Arbequina olive oil	30.4	47.6	231.2	73.6	39.0	61.0
California-Koroneiki olive oil	33.2	48.5	284.5	95.2	40.6	59.4
France-Nyons olive oil	34.1	49.2	285.5	94.0	40.9	59.1
Almond oil	*33.1*	*48.4*	*306.0*	*94.1*	*40.6*	*59.4*
Canola oil	33.1	49.3	321.0	102.2	40.2	59.8
Castor oil	*21.6*	*65.6*	*78.6*	*25.1*	*24.8*	*75.2*
Corn oil	*32.5*	*46.4*	*349.3*	*106.5*	*41.2*	*58.8*
Flax oil	29.3	47.4	437.7	123.0	38.2	61.8
Grape seed oil	33.6	46.8	359.7	108.8	41.8	58.2
Peanut oil	33.3	49.3	300.1	97.3	40.3	59.7
Sesame oil	*33.0*	*47.2*	*329.0*	*101.8*	*41.1*	*58.9*
Soybean oil	32.8	48.0	384.8	115.9	40.6	59.4
Sunflower seed oil	33.2	47.9	351.1	104.1	40.9	59.1
Oil mixtures						
Arbequina olive oil : almond oil (v/v)						
2:1	32.3	47.2	302.1	98.0	40.6	59.4
1:1	32.2	47.6	302.3	96.5	40.4	59.6
1:2	32.1	47.4	311.3	98.2	40.4	59.6
Arbequina olive oil : canola oil (v/v)						
2:1	31.8	50.0	303.7	95.0	38.9	61.1
1:1	31.9	50.7	307.1	94.7	38.6	61.4
1:2	32.0	49.9	306.1	95.3	39.1	60.9
Arbequina olive oil : castor oil (v/v)						
2:1	29.9	49.2	227.8	68.3	37.8	62.2
1:1	27.5	51.5	201.4	55.3	34.8	65.2
1:2	24.1	54.0	165.7	46.5	30.9	69.1
Arbequina olive oil : corn oil (v/v)						
2:1	32.1	48.4	277.1	88.2	39.9	60.1
1:1	32.5	48.4	306.2	96.1	40.2	59.8
1:2	32.1	48.1	308.5	95.3	40.0	60.0
Arbequina olive oil : flax oil (v/v)						
2:1	30.3	48.7	332.5	103.5	38.4	61.6
1:1	29.6	48.8	370.6	111.8	37.8	62.2
1:2	28.9	49.1	377.1	109.2	37.1	62.9
Arbequina olive oil : grape seed oil (v/v)						
2:1	32.0	47.5	327.2	108.8	40.3	59.7
1:1	31.9	47.8	334.5	103.2	40.0	60.0
1:2	33.1	48.2	320.5	98.7	40.7	59.3
Arbequina olive oil : peanut oil (v/v)						
2:1	32.3	48.6	283.1	90.9	39.9	60.1
1:1	33.2	50.6	285.1	91.0	39.6	60.4
1:2	32.9	49.7	295.8	94.9	39.8	60.2

TABLE 2. (Continued)

| Pure oils	A_{21}	A_{22}	T_{21} (ms)	T_{22} (ms)	% A_{21}	% A_{22}
Arbequina olive oil : sesame oil (v/v)						
2:1	33.0	47.8	329.0	101.8	40.8	59.2
1:1	32.3	48.3	288.8	91.1	40.1	59.9
1:2	32.3	47.5	303.5	95.1	40.5	59.5
Arbequina olive oil : soybean oil (v/v)						
2:1	32.1	49.3	308.9	96.9	39.4	60.6
1:1	31.7	49.1	320.3	98.5	39.2	60.8
1:2	32.8	50.1	347.0	105.6	39.6	60.4
Arbequina olive oil : sunflower seed oil (v/v)						
2 : 1	32.6	48.6	299.9	95.3	40.1	59.9
1 : 1	31.0	47.2	277.4	85.4	39.6	60.4
1 : 2	31.8	47.3	320.0	98.5	40.2	59.8
Koroneiki olive oil : almond oil (v/v)						
2:1	32.3	50.4	308.6	96.8	39.1	60.9
1:1	31.8	49.9	313.2	96.0	38.9	61.1
1:2	32.6	50.1	302.3	92.6	39.4	60.6
Koroneiki olive oil : castor oil (v/v)						
2:1	33.3	48.8	234.9	62.4	40.6	59.4
1:1	28.6	51.7	196.1	45.9	35.6	64.4
1:2	26.2	55.1	156.2	37.6	32.2	67.8
Koroneiki olive oil : corn oil (v/v)						
2:1	33.2	49.7	302.6	96.2	40.0	60.0
1:1	32.9	49.4	314.6	98.2	40.0	60.0
1:2	33.0	49.3	325.7	99.9	40.1	59.9
Koroneiki olive oil : sesame oil (v/v)						
2:1	32.1	51.5	299.1	91.0	38.4	61.6
1:1	31.7	50.1	337.9	102.6	38.8	61.2
1:2	32.6	50.6	325.5	97.7	39.2	60.8
France (Nyons) olive oil : almond oil (v/v)						
2:1	32.1	51.5	299.1	91.0	38.4	61.6
1:1	31.7	50.1	337.9	102.6	38.8	61.2
1:2	32.6	50.6	325.5	97.7	39.2	60.8
France (Nyons) olive oil : castor oil (v/v)						
2:1	34.3	49.5	213.7	57.9	40.9	59.1
1:1	31.5	49.9	188.9	45.0	38.7	61.3
1:2	25.3	55.2	164.0	36.7	31.4	68.6
France (Nyons) olive oil : corn oil (v/v)						
2:1	32.6	50.0	296.7	93.8	39.5	60.5
1:1	29.9	48.7	322.5	100.3	38.0	62.0
1:2	33.0	49.1	317.9	97.5	40.2	59.8
France (Nyons) olive oil : sesame oil (v/v)						
2:1	32.9	49.2	308.2	97.8	40.1	59.9
1:1	32.9	49.1	311.3	97.9	40.1	59.9
1:2	33.0	48.9	308.4	95.7	40.3	59.7
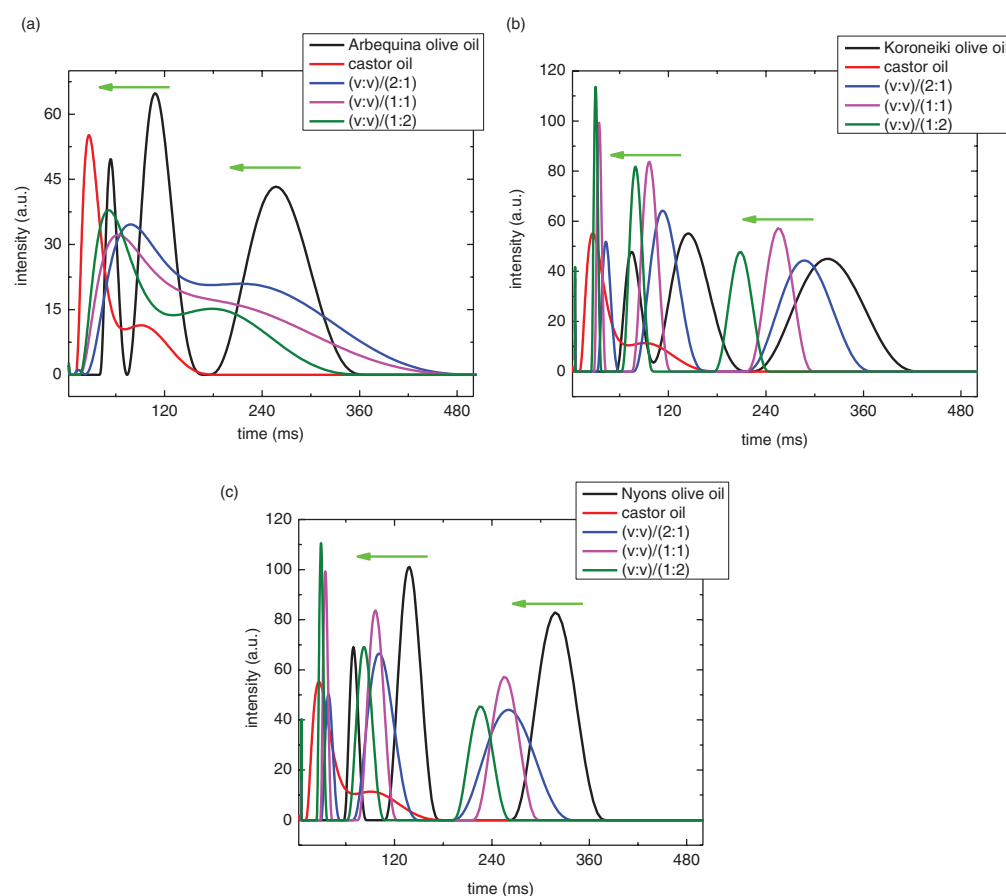
FIGURE 2 (a-c) Continuous T2 relaxation time data belonging to: Arbequina olive oil, castor oil, and their mixtures (a); Koroneiki olive oil, castor oil, and their mixtures (b); Nyons olive oil, castor oil, and their mixtures (c).

olive oil by T_2 relaxation distributions. In addition, as shown in Figure 2(b,c), both of the two signals of Koroneiki and France-Nyons olive oils around 70 ms and 140 ms started shifting towards the pure castor oil peak at 25 ms. The signal at 318 ms of the two olive oils also shifted towards 93 ms of the pure castor oil. Besides T_2 distribution analysis of olive oils with castor oil, as indicated in the supporting figures, T_2 distribution curves of the three olive oils in mixtures with almond, corn, and sesame oils were analyzed (see Figures 3, 4, and 5 showing T_2 distributions of olive oil samples with almond, corn, and sesame oils). In the T_2 distribution curves belonging to the mixtures of Arbequina olive oil and almond oil, T_2 peaks of olive oil broadened and overlapped with the T_2 peaks of the pure almond oil. In the case of Koroneiki and France-Nyons olive oils, the intensity of the T_2 peak at 318 ms belonging to the pure olive oils decreased, and started shifting towards higher values. In the mixtures of Arbequina olive oil with either corn or sesame oil (Figures 4 and 5), the number of T_2 peaks of pure Arbequina olive oil decreased to two. Both of these two peaks also broadened. The one around 310 ms started shifting towards the 350 ms

peak of the pure corn and sesame oils. When either Koroneiki or France-Nyons olive oil was mixed with either corn or sesame oil, the number of T_2 peaks remained the same before and after preparing the mixtures. The two peaks at around 70 ms and 140 ms did not show a significant change in their positions in the T_2 distribution curves of the mixtures with respect to the curves of the pure state. However, the third peak around 318 ms of Koroneiki and France-Nyons olive oils did not indicate a systematic shift towards the peak of pure sesame oil at around 310 ms.

Upon careful analysis and comparison of T_2 distributions for each pair of olive oil and either of the almond, castor, corn, and sesame oils, the continuous T_2 distribution curves obtained by Inverse Laplace transformation and belonging to the mixtures prepared by mixing Arbequina olive oil with either canola, peanut, or soybean oil as representative data (Figure 6 (a-c)). Firstly, the number of T_2 peaks in the T_2 distribution curves of Arbequina olive oil remained the same in the curves of the mixtures of Arbequina olive oil with either the canola, peanut, or soybean oil. Secondly, the mixtures of Arbequina olive oil with either peanut or soybean
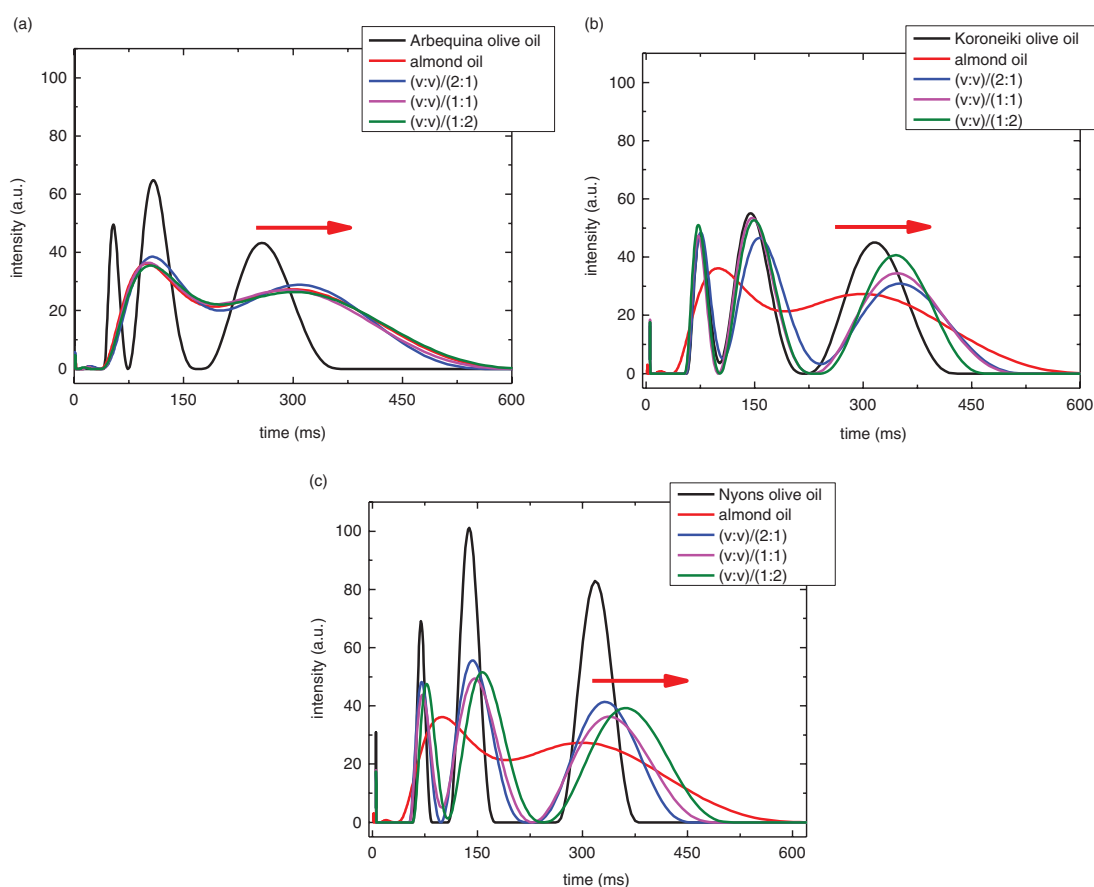
FIGURE 3   (a-c) Continuous T2 relaxation time data belonging to Arbequina olive oil, almond oil, and their mixtures (a); Koroneiki olive oil, almond oil, and their mixtures (b); Nyons olive oil, almond oil, and their mixtures (c).

oil yielded practically the same T_2 distributions. On the other hand, the appearance of T_2 distributions of the mixtures of Arbequina olive oil and canola oil look similar to those of the mixtures of Arbequina olive oil with either peanut or soybean oil. However, the T_2 distributions of the mixtures of Arbequina olive oil and canola oil had T_2 peaks with lower intensity and broader distribution. These T_2 distribution results in the case of the mixtures of Arbequina olive oil with either canola, peanut, or soybean oil clearly indicated that the possible adulteration detection needs to be further confirmed by other techniques such as UV-Vis spectroscopy.

The UV activity of olive oil arises from two types of natural pigments: chlorophylls and carotenoids (Minguez-Mosquera, 1997). The green hues of the oils are attributed to the existence of chlorophyll pigments, while the yellow hues come from carotenoids. Further, chlorophyll pigments are composed of tetrapyrole macrocycle and several conjugated double bonds, while carotenoids are isoprenoid compounds having a hydrocarbon structure with conjugated double bonds. The conjugated double bonds are responsible for the

absorption in the visible region of the spectrum of these segments (Moyano *et al.*, 2010). For example, the specific extinction coefficient at 268 nm corresponds to the maximum absorption of the conjugated trienes (Passaloglou-Emmanouilidou, 1990). There are several derivatives of chlorophyll pigments such as chlorophyll a, chlorophyll b, pheophytins, chlorophyllides, and pheophorbides (Minguez-Mosquera, 1997). There were no quantitative differences in the chlorophyll pigment percentage of the olive oils obtained from different olive cultivars produced in various countries (Giuliani *et al.,* 2011). Some researchers also identified qualitative differences in olive oil in terms of evidence of chlorophyll pigments (Cerretani *et al.*, 2008; Criado *et al.*, 2007). UV-Vis spectral results were also combined with a chemometric analysis (Torrecilla *et al.*, 2010 (a)), and multivariate calibration (Fuentes *et al.*, 2012). In the current contribution UV-Vis spectroscopy was employed as a qualitative and complementary tool to the LF[1]H NMR method. Figure 7 compares the UV-Vis spectra of pure Arbequina olive oil, castor oil, and mixtures of the two oils with (v:v/1:1).
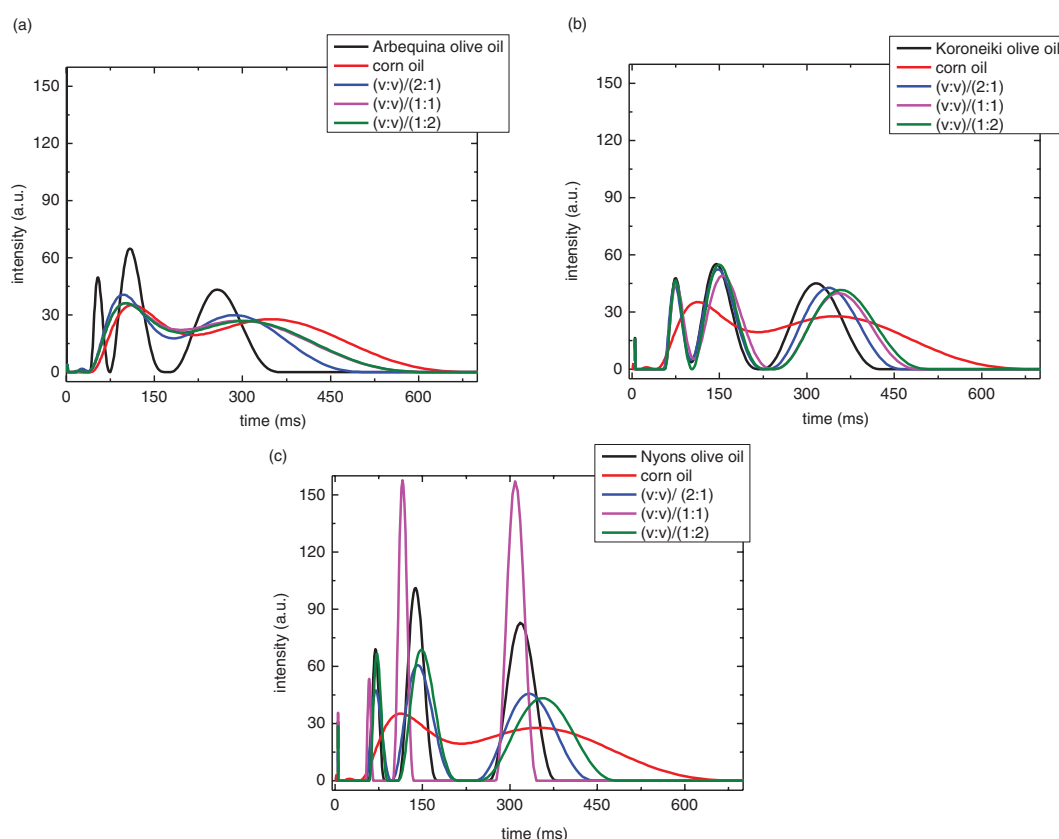
FIGURE 4   (a-c) Continuous T2 relaxation time data belonging to Arbequina olive oil, corn oil, and their mixtures (a); Koroneiki olive oil, corn oil, and their mixtures (b); Nyons olive oil, corn oil, and their mixtures (c).

The maximum absorbance values (λ_{nm}) of the UV-Vis spectra of the oils either in pure state or in mixtures with olive oil are also given (Table 3).

The common maximum absorbance values in the UV-Vis spectra are approximately at 244.0 nm and attributed to elenolic acid, a compound significant in concentration in the phenolic fraction of olive oil, and 282.0 nm to phenolic compounds (tyrosol, hydroxytirosol, and tocopherols) (Fuentes *et al.*, 2012). The oils other than olive oil have a common peak at around 272.0 nm arising from syringic acid, which is a phenolic compound, while the peak around 260.0–265.0 nm and observed in the spectra of Koroneiki, France-Nyons olive oils and in castor, corn, soybean and sunflower seed oils was explained by the presence of phenolic compounds, vanilic acid, and protocatechuic acid (Zhang *et al.*, 2013). Flax seed oil has a unique band around at 320.0 nm, which is linked to the existence of some flavonoids like apigenin and luteolin (Lerma-Garcia *et al.*, 2009). The unique peak of canola and soybean oils at 298.0 nm was explained by the existence of sinapic acid (Khattab *et al.*, 2010). The unique peak of olive oil with lower intensity around 414.0 nm was explained by the presence of pheophorbide b, which is a chrolophyll derivative,

and the peak at 660.0 nm was attributed to chlorophyll a (Roca *et al.*, 2010; Giuliani *et al.*, 2011). The differences in the maximum absorbance of the UV-Vis spectra of the oil blends with respect to their pure state show that UV-Vis spectroscopy is useful in detecting adulteration in olive oil.

Based on the UV-Vis spectral assignments, it is possible to suggest that in the case of possible adulteration of olive oil samples with almond oil, the unique maximum absorbance of almond oil at 273.0 nm shows the adulteration qualitatively. In addition to the adulteration detection of castor oil added olive oil by LF-NMR, UV-V is a signal of castor oil at around 273.0 nm and will also prove the adulteration of olive oil with castor oil. Similar to these cases, the addition of corn oil into olive oils might be detected by UV-Vis spectroscopy with the presence of the signal around 273.0 nm. In the case of the mixtures prepared by mixing an olive oil sample with sesame oil, the maximum absorbance values of olive oil and sesame oil in the region between 260.0 nm and 285.0 nm emerged, and yielded one peak in that region. This also helps with the possible adulteration detection of olive oil when the olive oil is mixed with sesame oil. Then the UV-Vis spectra of Arbequina olive oil mixtures
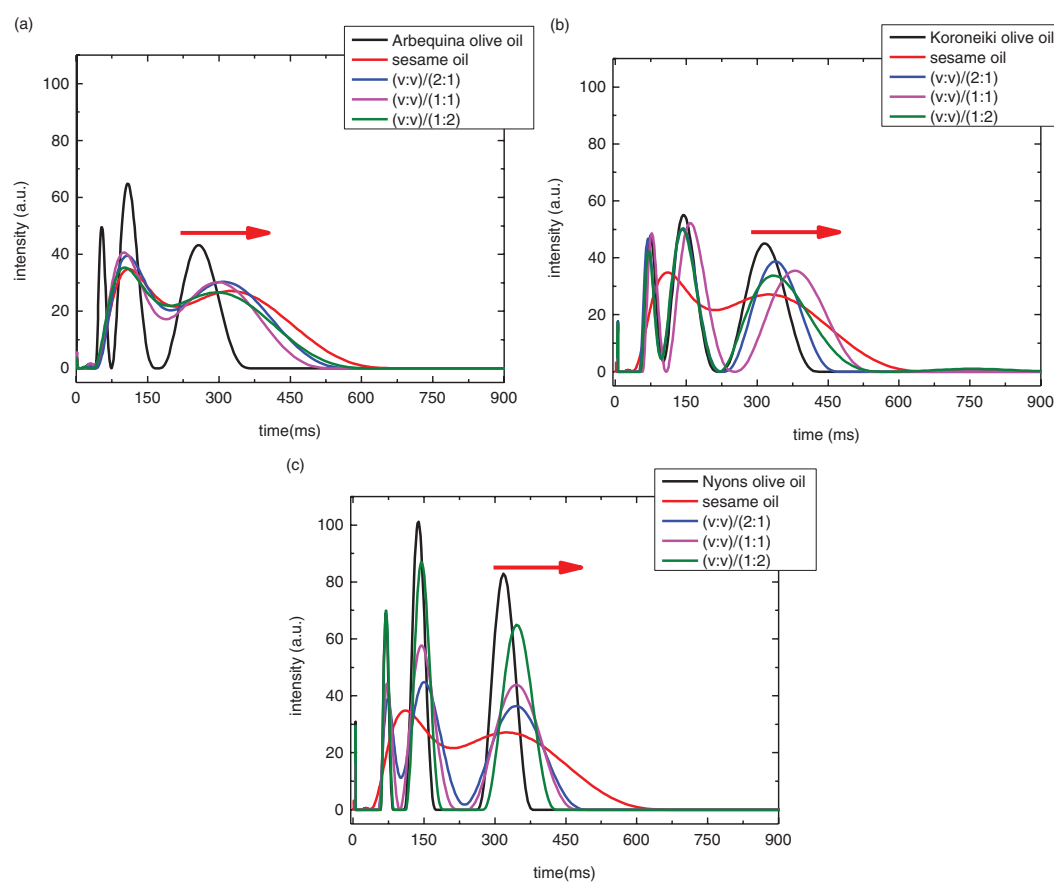
FIGURE 5 (a-c) Continuous T2 relaxation time data belonging to Arbequina olive oil, sesame oil, and their mixtures (a); Koroneiki olive oil, sesame oil, and their mixtures (b); Nyons olive oil, sesame oil, and their mixtures (c).

with canola, flax, grape seed, peanut, and soybean oils were analyzed. When olive oil was adulterated with flax seed oil, it was possible to detect the adulteration with the unique maximum absorbance at 320.0 nm of flax seed oil UV-Vis spectrum. In the possible adulteration detection of olive oil upon mixing olive oil with grape seed oil and sunflower oil, the maximum absorbance at 272.0 nm belonging to grape seed and sunflower oils will be useful in detecting the possible adulteration. The two maximum absorbance values at 267.5 nm and 277.0 nm of soybean oil UV-Vis soybean oil UV-Vis spectrum might be also utilized in proving the adulteration of olive oil samples. The unique maximum absorbance of canola and soybean oils at 298.0 nm makes it easy to detect the adulteration of olive oil when olive oil is mixed with either canola or soybean oil.

4. DISCUSSION

One of the major issues related to adulteration detection of natural products such as olive oil is intrinsic variability of both the examined olive oils and the adulterant oils. The intrinsic parameters such as free fatty acids (Nunes, 2014) of edible oils could depend on different factors including geographical origin. In particular for determining the minor constituents of edible oils with less than 1–2%, isolation might be needed (Zamora et al., 2001). However, in the current study, even the different oleoyl acyl contents of different olive oils, California-Arbequina and France-Nyons olive oils namely, were reflected in the T_2 decaying curves. Different properties such as viscosity of the adulterant oils led also to different T_2 relaxation times as shown in Table 2. Besides these, it is possible to detect the colorant molecules of both the olive oil samples and the other oils directly with UV-Vis spectroscopy without any isolation. Therefore, combining LF ^{1}H NMR and UV-Vis spectroscopy does not require focusing on the intrinsic variability of the oils, in particular the minor components while analyzing the adulteration detection.

There have been various contributions regarding the detection of olive oil adulteration by several techniques. For example, Mendes et al., (2015) studied near-infrared (IR) and mid-IR in addition to Raman spectroscopy in detecting olive oil adulteration with soybean oil of several adulterant
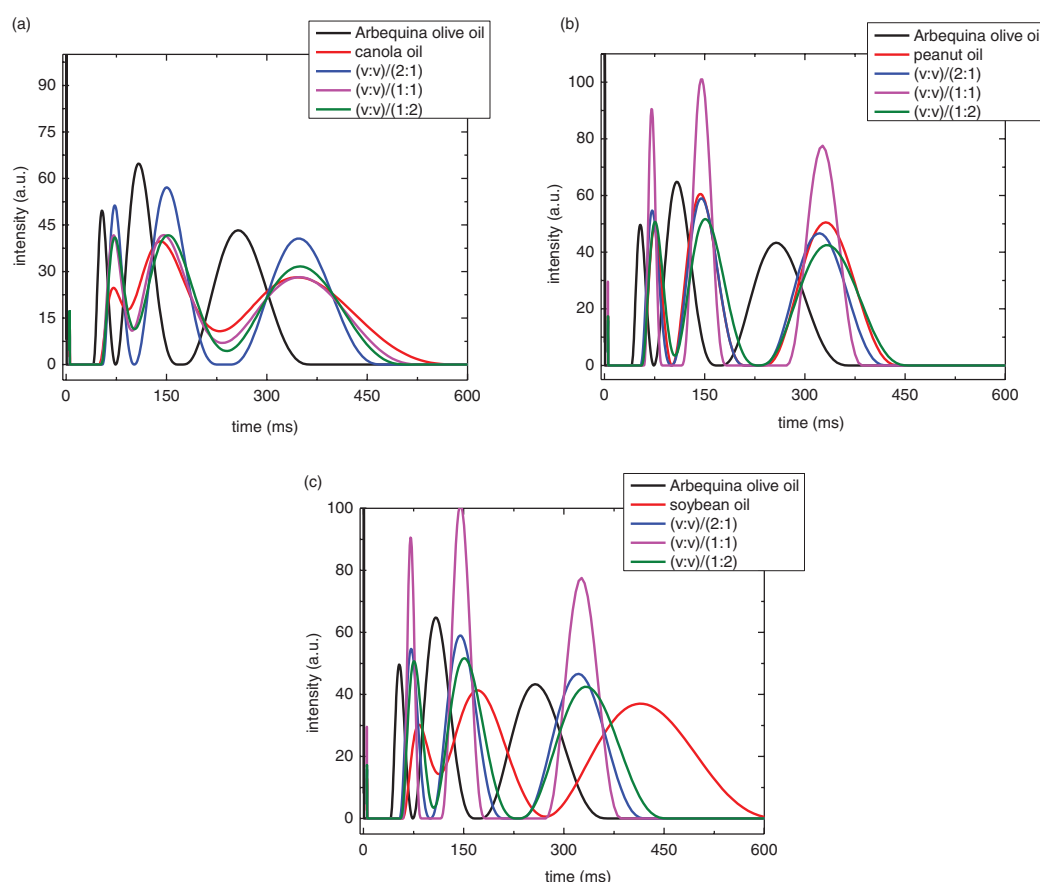
FIGURE 6.   Continuous T2 relaxation data of Arbequina olive oil, and its mixtures with canola oil (a); peanut oil (b); soybean oil (c).
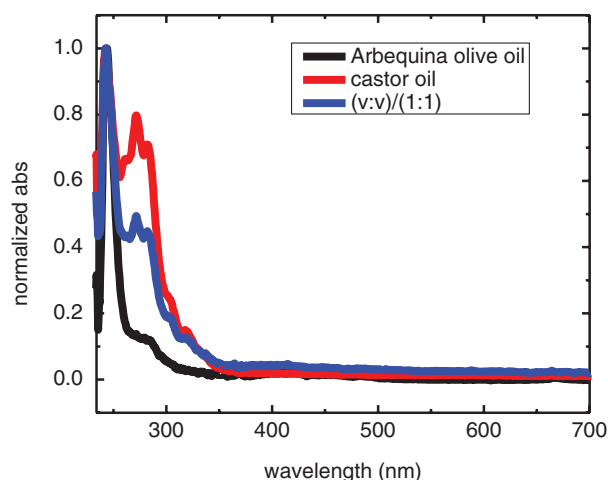

FIGURE 7.   The representative UV-Vis spectra of pure olive oil, pure castor oil, and the blends of them.

levels from zero to 100%, while Gurdeniz and Ozen (2009) focused on combining chemometric analysis with mid-IR for analyzing adulteration of olive oil samples mixed with either rapeseed, cottonseed,

and a corn-sunflower binary mixture. Further, Yang and Irudayaraj (2001) also combined near-IR, fourier-transformation (FT)-IR, and FT-Raman techniques for determining pomace olive oil adulteration in extra virgin olive oil. Rohman and Che Man (2010) used FT-IR for olive oil adulteration detection but mainly by mixing olive oil with palm oil. Among such contributions, Gurdeniz and Ozen (2009) showed that the manipulation of mid-IR spectra with chemometrics led to the detection and the quantification of olive oil adulteration. They also found that the detection limit for the adulterant oil or the binary oil mixture was at 5%. In the study by Smejkalova and Piccelo (2010), the potential of a high-power pulse-field gradient (PFG) NMR probe was used for detecting adulterated extra virgin olive oils. PFG NMR was employed for analyzing olive oil with adulterant oils of soybean, sunflower, peanut, and hazelnut. It was shown that combining PFG NMR with a multivariate classification method resulted in successful discrimination of authentic and high adulterated extra virgin olive oil with hazelnut oil (as low as 30%), peanut oil (as low as 30%), with soybean oil (as low as 10%), and sunflower oils (as low as 10%). Agiomyrgianaki

TABLE 3.   Maximum absorbance values of the UV-Vis bands of the oils of interest

	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)	λ_5 (nm)	λ_6 (nm)
Pure oils						
Arbequina-California	244.0	281.0	411.5	664.0	-	-
Koroneiki-California	243.0	264.5	403.0	664.0	-	-
France (Nyons)	245.5	265.0	400.0	660.5	-	-
Almond oil	244.0	273.0	284.0	-	-	-
Canola oil	245.0	278.5	298.0	-	-	-
Castor oil	244.0	260.0	271.0	282.0	-	-
Corn oil	244.0	260.0	272.0	282.0	-	-
Flax seed oil	272.0	282.0	320.0	-	-	-
Grape seed oil	262.0	272.0	282.0	-	-	-
Peanut oil	243.5	269.5	-	-	-	-
Sesame oil	244.0	272.0	284.0	-	-	-
Soybean oil	243.0	265.5	278.0	298.0	-	-
Sunflower seed oil	250.0	261.0	272.0	283.0	-	-
Oil Mixtures (v:v/1:1)						
Arbequina-California: almond oil	244.0	273.0	284.0	410.0	661.0	-
Arbequina-California: canola oil	245.0	276.0	298.0	401.5	660.5	-
Arbequina-California: castor oil	244.0	260.0	271.0	282.0	410.0	664.0
Arbequina-California: corn oil	244.0	260.0	272.0	282.0	410.0	664.0
Arbequina-California: flax seed oil	272.0	282.0	320.0	410.0	664.0	-
Arbequina-California: grape seed oil	262.0	272.0	282.0	410.0	665.0	-
Arbequina-California: peanut oil	244.0	266.5	276.0	398.5	663.0	-
Arbequina-California: sesame oil	244.0	272.0	411.0	664.5	-	-
Arbequina-California: soybean oil	247.0	267.5	277.0	298.0	398.5	664.0
Arbequina-California: sunflower seed oil	245.0	272.0	283.0	412.5	664.0	-
Koroneiki-California: almond oil	247.5	264.0	272.5	376.5	662.5	-
Koroneiki-California: castor oil	242.0	265.5	275.0	399.5	661.5	-
Koroneiki-California: corn oil	244.0	261.5	275.0	403.0	662.0	-
Koroneiki-California: sesame oil	247.5	277.0	662.5	-	-	-
France (Nyons): almond oil	245.5	272.5	282.0	657.0		-
France (Nyons): castor oil	243.0	262.0	274.5	308.0	658.0	-
France (Nyons): corn oil	244.0	256.5	277.0	659.5	-	-
France (Nyons): sesame oil	248.0	280.5	661.0	-	-	-

et al. (2010) also focused on the detection of olive oil adulteration with hazelnut oil by applying ^{1}H and ^{31}P NMR techniques in conjunction with multivariate statistical analyses of canonical discriminant analysis (CDA) and classification binary trees (CBTs). Both of the statistical techniques were successful in grouping the adulterated samples correctly. Xu *et al.* (2014) used LF ^{1}H unilateral NMR for the detection of virgin olive oil adulteration where olive oil was mixed with sunflower and red palm oils. Xu and co-workers measured the transverse relaxation and diffusion coefficients of different adulterated oils in analyzing the adulteration detection of olive oil.

There are several knowledge gaps in the literature studies mentioned above. Firstly, the number of olive oil and edible oil mixtures analyzed in these studies was limited. Secondly, the number of studies on the adulteration detection of olive oil when it is mixed with either castor oil or flax seed oil is scarce. The current report fills this gap of scarce data on adulteration detection of olive oil when the adulterant oil could be either castor or flax seed oil. Furthermore, it is important to show the adulteration detection of olive oil when the adulteration is at economically significant levels. In the present study, the possibility of olive oil adulteration detection is also shown in particular when an olive oil

sample is mixed with other edible oils at economically significant ratios. Moreover, in the techniques such as FT-IR, there is not a significant difference in the signals of pure and adulterated olive oils. For example, signals of C=O and C-H may arise from organic constituents of any edible oil. Hence, FT-IR signals of olive oil and the adulterant oil will overlap even at lower levels of adulteration. This clearly shows the need for finding representative signals by methods such as LF ^{1}H NMR in adulteration detection of olive oil. Besides filling such knowledge gaps in olive oil adulteration detection studies, it is also essential to use complementary methods rather than techniques which provide similar information.

In the present study, the detection of adulteration up to 66% with, for instance, castor oil was shown. This limit has significance because it is much higher than the economically significant level. This demonstrates that LF ^{1}H NMR has the advantage of detecting adulterated olive oil where the adulterant oil has an economically important fraction in the mixture. This might be defined as lower critical limit (LCL) for adulterated olive oil detection in an economic sense. Below this limit, adulteration perhaps will not be economically preferable. In other words, the employed method needs to determine the adulteration of olive oil above the lower critical limit. The obtained results by LF ^{1}H unilateral NMR showed detection of adulterations of olive oil with percentages of at least 10% of sunflower and red palm oils (Xu et al. (2014)). In the present study, the number of oils used for the adulteration study is higher than the number of adulterant oils mentioned in the NMR studies (Smejkalova and Piccelo, 2010; Agiomyrgianaki et al., 2010; Xu et al., 2014). It seems that measuring T_2 by LF ^{1}H NMR and determining continuous T_2 distribution help with olive oil adulteration detection where the adulterant oil percentage is economically significant. Because LF ^{1}H NMR is a non-destructive method, samples of LF ^{1}H NMR measurements could be used for further analysis by other methods. Besides this, it is also possible to compare and combine the results of LF ^{1}H NMR with the results obtained by UV-Vis in detecting the adulteration of olive oil.

5. CONCLUSION

The detection of olive oil adulteration was shown by LF ^{1}H NMR relaxometry and UV-Vis spectroscopy upon blending three olive oil samples having different oleoyl acyl contents with various edible oils. Bi-exponential fitting of the T_2 data indicate that difference in oleoyl acyl contents of olive oil samples is an important factor in following representative unique signals of adulterated olive oils. When the three olive oil samples were mixed with castor oil, both T_{21} and T_{22} values became shorter as the volumetric ratio of olive oil to castor oil was

changed from 2:1 to 1:1, and even further to 1:2. This was independent of which olive oil was used for preparing the mixtures. T_2 relaxation values became longer as the volumetric ratios of the olive oils to corn oil were increased. However, the two values did not become as long as the ones belonging to the pure corn oil. Furthermore, UV-Vis spectroscopy was useful in detecting the adulteration of olive oil qualitatively. For example, when olive oil is adulterated with flax seed oil, it is possible to detect adulteration with the unique maximum absorbance at 320.0 nm of flax seed oil UV-Vis spectrum. In the possible adulteration of olive oil with either canola or soybean oil, the UV-Vis band around 298.0 nm of the canola and soybean oils might help with determining the adulteration. It seems that the application of LF ^{1}H NMR relaxometry by itself will not be sufficient to detect the adulteration of olive oil when an olive oil sample is mixed with almond, sesame, and grape seed oils in particular. It should also be emphasized that although LF ^{1}H NMR is an easy method to operate, and in the current study LF NMR technique was employed in the adulteration detection of only three olive oil samples: California-Arbequina olive oil, California-Koroneiki olive oil, and France-Nyons olive oil. A higher of number of olive oil samples with different oleoyl acyl contents should be analyzed by the LF NMR technique in order to correlate, for example, T_2 decaying curves to oleoyl acyl contents which might range from 65% up to 85%. This might be useful in the adulteration detection of olive oil samples with different geographical origins. Combining non-destructive techniques of LF ^{1}H NMR and UV-Vis spectroscopy will be reliable and beneficial in detecting the adulteration of olive oil, which is not only an economic fraud but also can cause serious health problems. Combining such two different techniques also has the advantage of detecting the adulteration of olive oil above the lower critical limit (LCL) in an economic sense. Both methods have advantages of being easy to operate and non-destructive. Therefore, both methods could also be easily employed in controlling the virginity and purity of olive oil samples when olive oils are blended with some edible oils with different ratios.

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