

Thermal Deterioration of Virgin Olive Oil Monitored by ATR-FTIR Analysis of Trans Content

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The monitoring of frying oils by an effective and rapid method is one of the demands of food companies and small food retailers. In this work, a method based on ATR-FTIR has been developed for monitoring the oil degradation in frying procedures. The IR bands changing during frying in sunflower, soybean, and virgin olive oils have been examined in their linear relationship with the content of total polar compounds, which is a preferred parameter for frying control. The bands assigned to conjugated and isolated trans double bonds that are commonly used for the determination of trans content provided the best relationships. Then, the area covering 978–960 cm^{-1} was chosen to build a model for predicting polar material content for the particular case of virgin olive oil. A virgin olive oil was heated up to 94 h, and samples collected every 2 h constituted the training set. These samples were analyzed to obtain their FTIR spectra and to determine the composition of fatty acids and the content of total polar compounds. The excellent results predicting the polar material content (adjusted R^2 0.997) was successfully validated with an external set of samples. The analysis of the fatty acid composition confirmed the relationship between the trans content and the content of total polar compounds.

KEYWORDS: ATR-FTIR; olive oil; frying temperatures; trans fatty acids; total polar compounds

INTRODUCTION

Virgin olive oil is the most commonly used cooking oil in Mediterranean countries, where deep frying is a popular method for food preparation, especially in fast-food restaurants. Olive oil composition, with its low content of saturated fatty acids and high content of antioxidants (e.g., phenols), is very stable, making it an excellent oil to be applied in culinary processes that involves high temperatures such as frying and baking (1). Although some phenols, e.g. hydroxytyrosol and tyrosol-like substances, dramatically decrease in the first hours of frying, other phenols (lignans 1-acetoxypinoresinol and pinoresinol) remain in the oil after 25 h. In addition to its high stability, olive oil has a low melting point and it drains well from fried foodstuffs, which is a desirable property in frying oils. Therefore, the study of frying processes and new methods of frying monitoring for the particular case of olive oil is of major interest today, these studies also being driven by the increasing consumption of frying products.

Nearly half of lunch and dinner food orders in restaurants and canteens include one or more items fried with edible oils (2). During frying, due to the high temperature and absorption of oxygen and water, oil suffers a wide range of reactions, namely hydrolysis, oxidation, isomerization, and polymerization (3). These reactions result in significant changes in the oil: e.g., a rise in polar substances, the decomposition of essential fatty acids, and an increase in trans isomers (4) occur. The frying process must be carefully controlled, because the oil darkens and thickens over time and the final product may have an unpleasant acid

taste and odor. Aside from quality losses, the degradation compounds produced during frying have nutritional and toxicological implications, since they are absorbed by the food and ingested by consumers. In consequence, frying oils have to be replaced regularly before significant deterioration has taken place which may have negative flavor or toxicological consequences.

Many methods have been developed to guide frying oil turnover, including the determination of free fatty acids, total polar compounds, alkaline materials (soaps), Lovibond color, metals, anisidine value, peroxide value, and mono- and diglycerides (5). Of these, the determination of the total polar compounds (TPC) is generally considered the most appropriate method for the measurement of fat deterioration during frying (6), because TPC are well correlated with the oxidized fatty acids generated at frying temperatures (7). Thus, the determination of polar materials seems to be the method that has been adopted in most of the regulatory systems, the cutoff limit being around 25% of polar compounds, after which the frying oil should be discarded (8).

The official method for TPC analysis is based on the separation of polar compounds by column chromatography followed by gravimetric analysis, a tedious and time-consuming procedure. Although workable in a laboratory setting, it is totally impractical in restaurants and food service operations and there is great interest in developing faster determination of TPC (9). Thus, in home frying, restaurants, and food services the replenishment of the oil is merely based on changes in the physical properties of frying oils used as quality indicators. As a result of this rudimentary method, mostly based on subjective assessment, the official limit of 25% of polar compounds may be often exceeded. Aside from domestic frying, in deep-fat-frying industries the increasing

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consumption of edible oils and the relative high cost of frying oils in relation to the final product price have encouraged the companies to find new rapid, accurate, and cost-effective techniques of quality control.

Some rapid methods have been proposed to serve as alternatives to the lengthy official methods. Thus, some authors have reported chemosensor-based methods for frying control (10). However, this method still fails in reproducibility and repeatability and it cannot be validated to become a standard method. Physical methods, such as ultrasonic measurements of the oil, have been suggested to monitor the oil quality during frying (11). Nevertheless, measurements by this method highly depend on the oil temperature; therefore, this variable must be controlled in order to obtain repetitive and reliable results. Spectroscopic methods have also been proposed to survey frying oils for quality, being rapid, nondestructive, and easy to apply, providing varied chemical information in a single step without the use of hazardous reagents and solvents. UV spectroscopy can be used to detect conjugated dienes produced during frying (12). Other methods are nuclear magnetic resonance (NMR) (13), near-infrared (2, 14), and Fourier transform infrared spectroscopy (FTIR). The last method has been used for the quantitative determination of some parameters related with frying oils, such as free fatty acids (15), quantification of trans fatty acids (16), peroxide values (17), and iodine value (18, 19), although little work has been carried out in relation to frying oils per se.

Despite the great potential of FTIR in characterizing frying oils, not many studies have been addressed in this regard. Innawong et al. (20) developed a methodology to classify frying oils into different frying times by ATR-FTIR spectra and PCA, using whole spectra in lieu of functional group identification. On the other hand, Moya et al. (4) studied unsaturation and hydroperoxide changes in frying oils by using windows of potassium bromide to hold the sample. This study was based on polyunsaturated fatty acids, where the reduction of unsaturation and the production of hydroperoxides are remarkable under thermal stress (21). Other works include measurements of unsaturation degree and cis and trans double bonds (4, 22) and the use of FTIR for evaluating the quality of the final fried product (23).

Among all the sample handling accessories commonly used in FTIR, attenuated total reflectance (ATR) provides a better measurement of the major changes during frying in comparison with other types of spectroscopy (24). The ATR-FTIR analysis is very simple, and it only entails settling a nonmeasured amount of the neat oil onto the entire surface of the ATR crystal, which is easily cleaned afterward to be ready for the next analysis. The short effective path length of the ATR accessory allows one to analyze all the bands changing during frying, since none of them are off-scale (19, 25). Consequently, it is a suitable method to evaluate the IR bands or wavenumbers covering all of the medium-IR range to assess the quality alteration of frying oils as an alternative to polar compound determination or other official methods. The number of bounces of the IR beam within the ATR accessory determines the effective path length and the sensitivity in detecting spectral changes.

The purpose of this work was to study the possibilities of the FTIR-ATR technique to predict the percentage of total polar compounds in virgin olive oil heated at frying temperatures, select the best wavelength regions for predicting the quality parameters, and validate the prediction accuracy of the developed mathematic model. The study is first centered on sunflower, soybean, and virgin olive oil and, later on, is focused on virgin olive oil to build a mathematical model to track chemical alteration during thermal degradation at frying temperatures. Since the thermoxidation

behavior of oils depends in great extent on fatty acid composition, the latter was analyzed by GC to give chemical support to the results attained by ATR-FTIR and to assist in their interpretation.

MATERIALS AND METHODS

Oil Samples and Heating Procedure. All of the heated oil samples were collected from a 4 L domestic fryer that was set at $190 \pm 1^\circ\text{C}$ with the temperature monitored by using an electronic temperature control probe (Heidolph EKT 3001, Kelheim, Germany). The fryer container and the tools for sample collection were cleaned and dried thoroughly before each experiment. The samples collected from the fryer were stored under nitrogen in brown glass 60 mL vessels and refrigerated at 4°C .

For a preliminary study, three oils were purchased at retailers (virgin olive oil, sunflower oil, and soybean oil) and heated from 0 to 25 h, and samples were extracted every 5 h.

Further experiments were carried out with a sample of virgin olive oil that was heated to frying temperatures, and representative samples were extracted during the whole heating period. The heating procedure was carried out for 8 h per day up to 94 h. Samples of 40 mL were collected at 2 h cycles and kept at 4°C before being used for chemical analysis and spectral collection. For validation purposes, the experiment was repeated with other virgin olive oil from a different retailer under the same conditions.

The repeatability study was carried out by analyzing a sample of olive oil five times per day for 1 week by three untrained operators. The resulting data were processed and expressed as relative standard deviation (%RSD) to determine repeatability and intermediate precision according to time and operator, following the procedure described in ref 26.

Analysis of Total Polar Compounds (TPC). Total polar compounds (TPC) were determined gravimetrically on the basis of the IUPAC Standard Method (27). Starting from 1 g of oil, nonpolar and polar fractions were separated by silica gel column chromatography (20 g silica adjusted to a water content of 5%, w/w) using 150 mL of hexane–diethyl ether (90:10) and 150 mL of diethyl ether as elution systems. The nonpolar fraction was eluted with 150 mL of *n*-hexane–diethyl ether (90:10, v/v), while the polar fraction was eluted with 150 mL of diethyl ether. The efficiency of the separation was confirmed by TLC using *n*-hexane–diethyl ether–acetic acid (80:20:1, v/v/v) and visualized with iodine vapor. The percentage of polar fraction was calculated by weighing both fractions after the evaporation of the solvents.

Fatty Acid Analysis. Fatty acid analysis was carried out by gas chromatography. The fatty acids were transmethylated according to the ISO norm (28). About 0.2 g of virgin olive oil was dissolved in 4 mL of hexane and 0.4 mL of a 2 N solution of KOH in methanol. The mixture was vigorously shaken for 10–15 s. The sample (0.2 μL) was injected into a gas chromatograph (Varian 3900) equipped with a split–splitless injector and a flame ionization detector. An SP-2380, 60×0.25 mm i.d. and 0.25 μm column (Supelco) was used. The injector and detector temperatures were set at 225 and 250 $^\circ\text{C}$, respectively. The oven temperature was kept at 170 $^\circ\text{C}$ for 10 min and then programmed from 170 to 210 $^\circ\text{C}$ at 1.5 $^\circ\text{C}/\text{min}$ and finally kept at the latter temperature for 8 min. Hydrogen was used as carrier gas at a flow rate of 1.0 mL/min. Each sample was analyzed in duplicate.

The results were expressed as percentages according to the total integrated area. The results were normalized according to the method described by Dobarganes et al. (29).

ATR-FTIR Spectroscopy. A Bruker 55 Equinox S FTIR spectrometer with a DGTS detector (Bruker Optics, Ettlingen, Germany) was used in this study. The sampling station was equipped with an overhead, detachable attenuated total reflectance (ATR, six bounces, Specac, Orpington, U.K.) accessory consisting of a zinc selenide crystal mounted in a shallow channel for the sample containment. Each spectrum was recorded at room temperature in the region of 4000–600 cm^{-1} by an average of 50 scans at a resolution of 4 cm^{-1} . Before each sample was scanned, the background spectrum was taken with an empty ATR crystal and stored in the computer. Each sample (240 μL) was spread uniformly through the ATR crystal and analyzed in duplicate. After the analysis, the ATR crystal was thoroughly cleaned with alcohol to eliminate the presence of oil/fat residues between measurements and then wiped with cotton. Spectra were manipulated with OPUS version 4.0 (Bruker Optics, Ettlingen, Germany).

Peak areas were computed on the raw spectra after ratioing against the background and the results exported as ASCII data for further statistical analysis.

Statistical Analysis. Univariate and multivariate algorithms have been applied by means of Statistica version 6.0 (Statsoft, Tulsa, OK). Stepwise multiple linear regression analysis (SMLRA) was the statistical procedure for the selection of fatty acids and IR bands that better correlate with the heating time or polar compound percentage. The objective of this statistical procedure was the design of a model in which the selected wavenumbers could predict how much an oil sample is altered after undergoing several frying procedures, using the TPC as reference index for assessing the alteration degree of the samples. The F-to-enter and F-to-remove values were specified a priori to select the wavenumbers to be included or excluded from the model (30). In order to ensure the significance of the results and avoid good results by chance, the F-to-enter/remove values were selected under the strictest conditions (Fisher distribution table at $p = 0.05$). Once the best variables were selected, a model for frying monitoring was obtained by linear regression analysis.

RESULTS AND DISCUSSION

Design of an ATR-FTIR Model for Frying Monitoring. Frying oils are subjected to a variety of chemical degradations, all of them occurring at the same time as a consequence of high temperatures. Thus, there are a variety of chemical parameters that may be monitored and used as an index of oil deterioration and quality as an alternative to the official TPC method. At the same time, the abundance of new chemical species is an inconvenience, since the complexity of the resulting chemical composition makes it difficult to select a single marker indicative of all the quality changes occurring in the oil. FTIR spectroscopy allows one to identify changes of chemical species of very different chemical nature in a single analysis by virtue of their functional groups. Nevertheless, not all spectral changes observed in the spectra of frying oils have the same significance and provide the same information on thermal degradation. Thus, with the purpose of selecting the IR bands that better inform of the frying practice, a set of oils were heated at frying temperatures (190 °C) and analyzed by FTIR spectroscopy. The samples were divided into monounsaturated (olive oil) and polyunsaturated oils (sunflower and soybean oils). This division is justified by their different fatty acid compositions, which greatly affects their thermoxidation kinetics and IR spectral properties (31). The percentage of total polar compounds (%TPC) analyzed in these samples showed values in the range 4.9–39.1% for soybean oil, 4.1–48.1% for sunflower oils, and 4.5–27.3% for virgin olive oil. The dissimilar behavior of oils, when they undergo the same thermal stress, is due to the composition of saturated, mono-unsaturated, and polyunsaturated fatty acids together with the concentration of minor compounds with antioxidant properties, mainly phenols and tocopherols. The samples of virgin olive oil produced the lowest TPC values, which support its suitability for frying oils because of its content of monounsaturated fatty acids and antioxidant compounds (1). On consideration of the %TPC as a dependent variable, the mathematical procedure of stepwise multiple linear regression analysis (SMLRA) was applied to the whole spectra (4000–600 cm^{-1}) to select the wavenumbers that provided the best regression results. **Table 1** shows the wavelengths selected when SMLRA was applied to each oil type. The relative standard deviations (%RSD) of the selected bands obtained in the repeatability study were lower than 10% in all cases, and therefore they were suitable variables for designing a calibration model. Except for the wavenumber of 725 cm^{-1} in polyunsaturated oils—assigned to a CH_2 rocking vibration and located near the band of the cis bond at $\sim 695 \text{ cm}^{-1}$ —all the selected bands were mostly located in the region assigned to trans double bonds (~ 970 and $\sim 980 \text{ cm}^{-1}$) and

Table 1. FTIR Wavelengths Selected by SMLRA When Regressing against Polar Compounds^a

wavelength (cm^{-1})	adjusted R^2	RSD% ^b
Monounsaturates (Olive Oil)		
970	0.99	5.48
972	0.99	5.52
974	0.98	5.55
1065	0.91	4.79
1082	0.74	4.02
1105	0.86	3.82
1217	0.92	4.35
1238	0.85	4.23
Polyunsaturates (Sunflower and Soybean Oil)		
725	0.97	2.65
951	0.94	5.75
974	0.99	5.55
989	0.99	5.52
1387	0.77	6.69

^aRegression coefficients higher than 0.95 are highlighted in boldface print.
^bRelative standard deviation.

the fingerprint region (1500–900 cm^{-1}) (19). It is noticeable that other IR bands that also change during frying were not selected, such as bands assigned to alcohols (3050 cm^{-1}), cis double bonds (3008 and 695 cm^{-1}) and free fatty acids (1716 cm^{-1}) (32). Although important changes were observed at these wavelengths, they did not correlate with the content of polar compounds, which shows that these IR bands follow a different evolution during thermal degradation.

The selected wavenumbers that provided better results in terms of adjusted R^2 ($R^2(\text{adj}) > 0.95$) in the monounsaturated oils (virgin olive oil) corresponded to the band attributed to the single trans double bonds ($\sim 970 \text{ cm}^{-1}$) (32). The increase of trans double bonds is explained by the isomerization of cis double bonds as a consequence of thermal treatment of the oils. In polyunsaturated oils (sunflower and soybean oils), in contrast, the SMLRA method selected wavenumbers located in the band of trans conjugated double bonds or trans conjugated with cis double bonds ($\sim 989 \text{ cm}^{-1}$), since the higher unsaturation of these oils makes this band rise during thermal degradation rather than the band assigned to single trans double bonds. This fact again reveals the significant variation in the chemical degradation of oils with different compositions. Therefore, if possible, a methodology for frying monitoring should address a particular oil type and frying temperature being used in the frying operation. A failure to do so may mean a misinterpretation of the degradation rate, and it may compromise both the quality and the safety of the oil (5).

The selection of wavenumbers related to trans double bonds points out the high correlation between the cis/trans isomerization of double bonds and the process of frying and the rise of polar materials. The utility of the IR trans bands for frying monitoring has the inconvenience that no single band elucidates the total trans double bonds in the sample. On the contrary, the spectral information on trans double bonds is split into two bands corresponding to single and conjugated trans double bonds. Each of these two bands has different extinction coefficients, and additionally, in ATR-FTIR analysis the effective path length is variable along the wavenumber (33), which means that the intensities measured at different wavenumbers are not comparable. Consequently, it is not possible to determine the total trans content by summing up the intensity of both bands. This inconvenience is added to the problems of trans quantification by IR

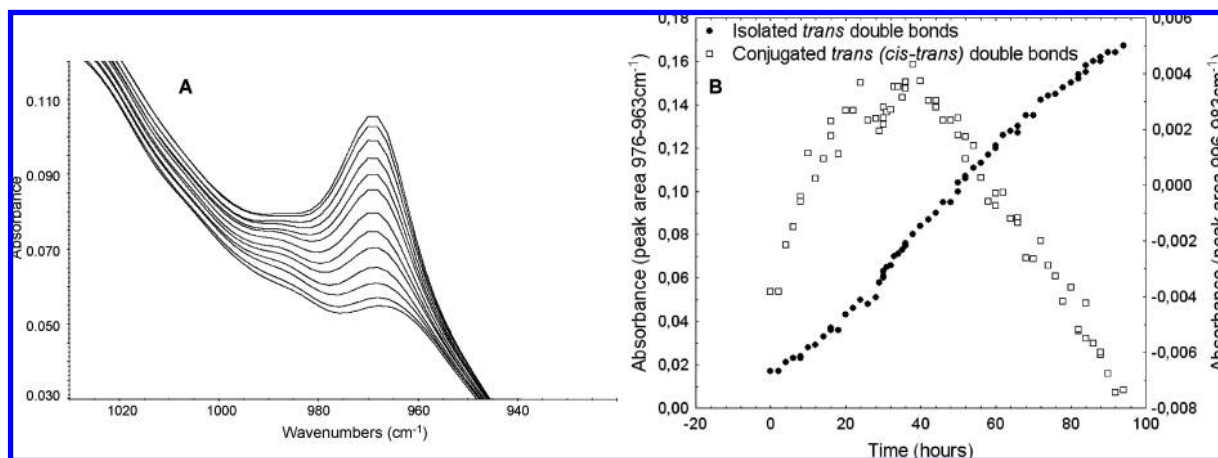


Figure 1. Spectra of the heated oils (A) and evolution of the peak areas of the bands assigned to conjugated (996–983 cm⁻¹) and isolated (976–963 cm⁻¹) trans double bonds (B).

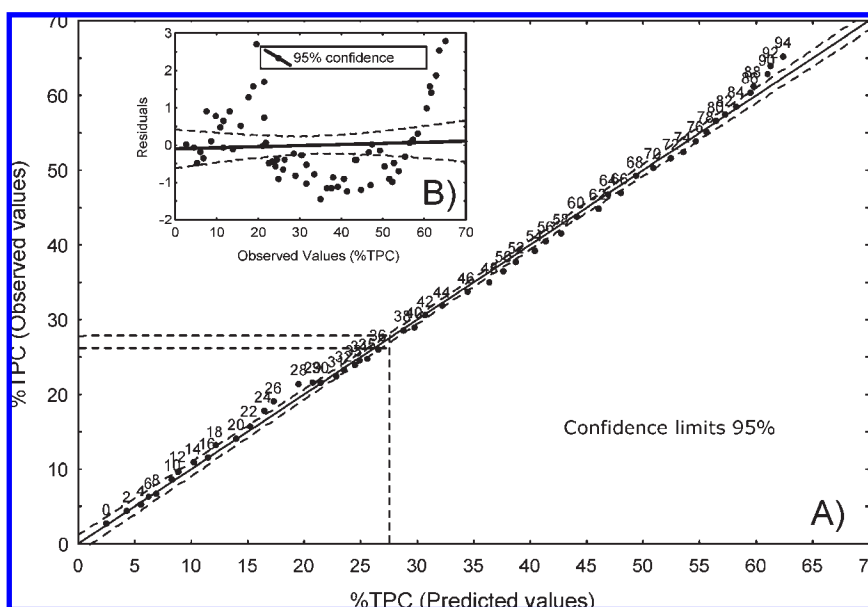


Figure 2. Plots of the percentage of total polar compounds (%TPC) and the values estimated from the equation obtained by regressing the %TPC and the area under the band at 967 cm⁻¹ (978–960 cm⁻¹) (A) and of observed values vs residuals (B).

because of the weak triacylglycerol absorptions in the trans region, which is ultimately affected by the fatty acid profile (19). The distribution of the trans double bonds into single and conjugated forms also depends on the fatty acid composition. In the particular case of olive oil, however, oleic acid is the major fatty acid, which accounts for about 85% of fatty acids (1), and therefore isolated trans double bonds are the most likely form to occur during frying. Under the assumption that the amount of conjugated trans double bonds is insignificant, and they are ultimately transformed into isolated trans double bonds, the measurement of the isolated trans bond band could serve as a monitoring variable for frying with olive oils. In order to prove this hypothesis, olive oil was heated in a domestic fryer at 190 °C and samples were collected sequentially every 2 h and analyzed on ATR-FTIR. The oil heating was carried out daily with one-night breaks in order to emulate the discontinuous frying that typically is implemented in restaurants and other small retail establishments, where uncontrolled variables such as differences in heating and cycles make the frying process difficult to control (34). **Figure 1A** shows the evolution of the trans double bonds computed in peak areas, for the isolated (978–960 cm⁻¹) and

conjugated (996–983 cm⁻¹) forms. The values for conjugated trans double bonds are much lower than those for the isolated form, due to the abundance of oleic acid compared with the low concentration of polyunsaturated fatty acids. On the other hand, the evolution of both forms of trans bonds clearly differs between them. Thus, although the low absorbance intensities of the band assigned to the conjugated form is close to the signal-to-noise ratio limit and therefore they are difficult to quantify, **Figure 1B** indicates a rise of them until 20–30 h, and then they shrink to basal levels. In contrast, the isolated conjugated double bonds linearly increase during the entire heating operation and these may be used to track the frying process. Therefore, the next study was to correlate the rise of this band with the values of %TPC, which also were determined in these samples. The peak area was considered the best parameter to measure the trans band, since a single peak height measurement may be strongly affected by the characteristic baseline tilt in the trans region as a consequence of underlying and adjacent absorptions (16). The integrated area covering the region 978–960 cm⁻¹ with two baseline points located at the cited wavenumbers yielded the best linear relationship with %TPC values. **Figure 2A** shows the calibration line

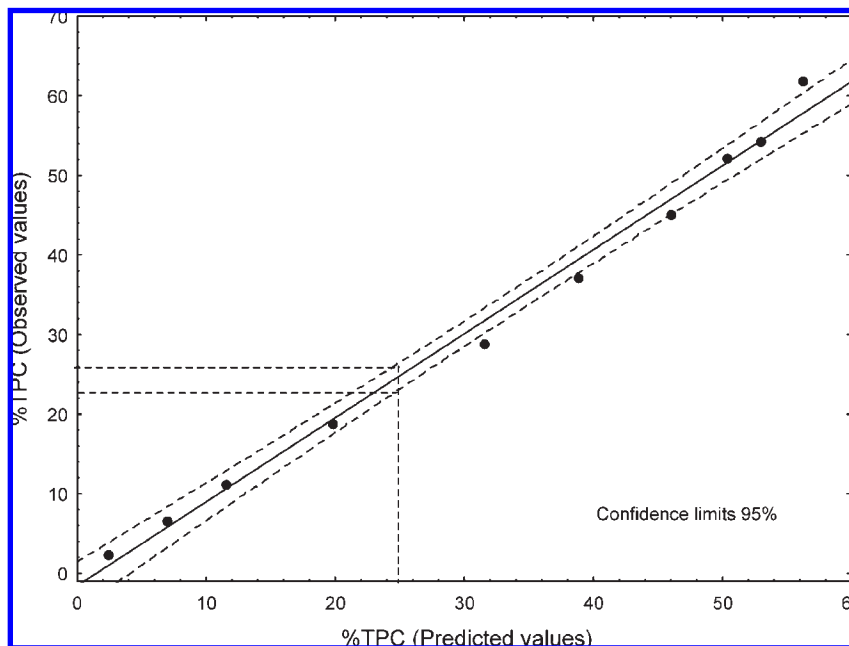


Figure 3. Plot of the percentage of total polar compounds (%TPC) and the values estimated from the equation obtained by regressing the %TPC and the area under the band at 967 cm^{-1} ($978\text{--}960\text{ cm}^{-1}$) in the validation set of samples.

obtained by simple regression analysis, whose equation (eq 1) is as follows.

$$\% \text{TPC} = -2.03 + 226.24 \times \text{peak area } 978\text{--}960\text{ cm}^{-1} \quad (1)$$

The high squared adjusted regression coefficient ($R^2(\text{adj}) = 0.997$) proves the high correlation of this band with the content of total polar compounds. Even though the IR band considered in this study is mainly due to the trans isomer of oleic acid and does not include those conjugated trans double bonds coming from other fatty acids, the regression analysis performed in olive oil samples proves that it can be used as an alternative to the measurements of polar materials or other lengthy analysis. The values of %TPC and the selected IR band area are also correlated with the time of heating, which indicates that both parameters increase over time and give information on the heating time. Thus, the linear regression analysis of %TPC and the IR band area versus the heating time (in hours) gave $R^2(\text{adj})$ coefficients of 0.999 and 0.994, respectively.

The residual analysis for eq 1 (**Figure 2B**) indicates a standard deviation of 1.034% of %TPC, with a maximum deviation value of 2.8%. However, the deviation from actual values seems to be related to the extent of heating time. Thus, although the regression equation allows one to estimate the %TPC with a low error, the predicted values may be overestimated when %TPC values are lower than 20% or higher than 60%, and they may be underestimated over other time frames. The value of 20% of %TCP, for instance, corresponds approximately to 20 h of heating, which indicates that this deviation may be due to the decomposition of conjugated trans double bonds into isolated form, as is shown in **Figure 1B**.

The applicability of the area under the trans IR band to frying monitoring depends on the repeatability of its measurement by ATR-IR as well as the repeatability of the evolution of this band over time when the heating operation is repeated. In order to evaluate the repeatability of the measurement, 105 spectra of a sample of olive oil were collected by three untrained operators over 7 days. The repeatability of the selected IR band area in terms of relative standard deviation (%RSD) was 2.5%, while

this value rose to 4.2% for the intermediate precision according to operator and time. As the %RSD values were lower than 10%, the next step was to check the capability of the equation in estimating %TPC values when it is applied to an external set of samples to test the robustness of the model. Furthermore, the validation of the model with a validation set of samples implies the study of the repeatability of the frying operation and the degradation mechanisms occurring when the procedure is repeated and the same type of oil and temperature are used. In the particular example of discontinuous frying the reproducibility of this process may be perturbed by some uncontrolled factors (34). For validation purposes, and also to confirm the observations of the previous experiment, a new sample of virgin olive oil was heated for 94 h, and 10 samples were collected and subsequently analyzed by ATR-FTIR. **Figure 3** shows the plot of the predicted values of %TPC vs the actual values. The %TPC values were predicted within $\pm 3\%$. Only the sample with the highest percentages of polar materials (62%) had 5% of error, which is due to the mentioned lack of linearity of the model for the highest values of %TPC (**Figure 2B**). In addition to the high complexity of all the chemical reactions occurring in the frying process, these results prove that the increase of trans double bonds in virgin olive oil is reproducible to an extent that can be used to predict polar compounds, even though the bands assigned to isolated trans double bonds are affected by the underlying process of triglycerides (16). As the trans IR band is subject to errors coming from the triglyceride underlying absorption, the good results obtained may be due to other chemical processes different from cis/trans isomerization that develop following the same evolution as heating time and %TPC and are somehow reflected in this band. In order to dismiss this possibility, the relationship between the percentage of polar compounds and trans content was confirmed by analyzing the fatty acid composition and tracking the trans double bonds during oil heating.

Study of Trans Double Bonds and Fatty Acid (FA) Composition of Virgin Olive Oil during Frying. The FA composition of the 54 samples used in the design of the model (eq 1) was determined by GC. The FA esters were obtained by using the alkaline esterification method, since it allows the quantitative determination of FAs

Table 2. Fatty Acid Composition (%) at the Beginning and End of the Frying Process^a

	(start value—end value)
C14:0	(0.01–0.02)
C16:1 <i>t</i>	(0.12–0.08)
C16:1 <i>ω</i> 9	(0.83–0.57)
C18:0	(3.88–4.11)
C18:1 <i>t</i>	(0.01–2.15)
C18:1 <i>ω</i> 9	(77.45–51.85)
C18:1 <i>ω</i> 7	(2.09–1.49)
C18:2 <i>tt</i>	(0.00–0.05)
C18:2 <i>ct</i>	(0.11–0.06)
C18:2 <i>ω</i> 6	(4.44–0.67)
C20:0	(0.40–0.41)
C18:3 <i>ω</i> 3	(0.73–0.01)
C20:1	(0.26–0.15)
C22:0	(0.11–0.11)
C24:0	(0.05–0.06)

^a Data have been normalized by the most abundant saturated fatty acid (C16:0).

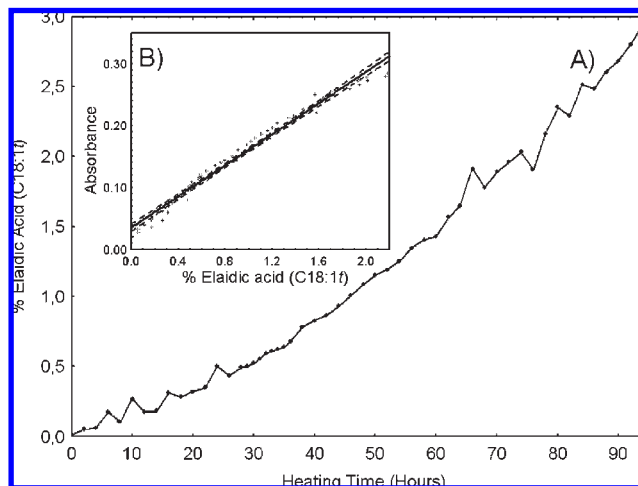
without any further chemical modification or artifact compounds that are different from those present in frying oils (35). The low acidity determined in the samples (0.54–0.85%) assured the applicability of this method as an alternative to the acid esterification method. **Table 2** shows the range of percentages for each of the fatty acids quantified in the samples. The chromatograms showed a steady decrease of linoleic and linolenic acids and an increase of elaidic acid, the trans isomer of oleic acid (C18:1*t*) (**Figure 4A**). Thus, the total content of trans fatty acids rose from 0.13% quantified in the original oil to 2.28% in the oil heated for 94 h. This end value of trans content is significantly lower than those reported by Grandirad et al. (36) for a sunflower oil heated at 240 °C for only 2 h, where the trans percentage accounted for only 5%. The difference in the content of polyunsaturates between olive oil (~15%) and sunflower oil (~86%) explains this lower value in the former oil. In order to know which fatty acids better correlate with the increase of polar compounds, SMLRA was applied to all the variables from the fatty acid analysis. The procedure, under the strictest conditions (F-to-enter for $F(F) = 0.975$ and tolerance 10–3), selected the elaidic, linolenic, and palmitoleic acids to be included in a predictor model. The resulting regression equation (eq 2) provides a regression coefficient ($R^2(\text{adj})$) of 0.997.

$$\% \text{TPC} = 12.24 + 19.14 \times [\text{C18:1}t] - 7.35 \times [\text{C18:2}\omega 6] + 27.52 \times [\text{C16:1}\omega 9] \quad (2)$$

The standardized coefficients proves that elaidic acid (0.661) has more influence on the equation than linoleic (−0.45) and linolenic acids (0.109). Thus, the relationship between the major trans fatty acid—elaidic acid—and %TPC is expressed as follows ($R^2(\text{adj}) = 0.984$; $\text{STD} = 0.024$).

$$\% \text{TPC} = 6.13 + 28.56 \times [\% \text{C18:1}t] \quad (3)$$

The results indicate that the concentration of elaidic acid is enough to predict the percentage of polar compounds, and therefore, the IR band assigned to isolated trans double bonds—mainly due to this acid—may be suggested for the monitoring of frying procedures with virgin olive oil. In order to verify the assignment of this band to trans double bonds, the concentrations of elaidic acid and the areas of the IR band were then regressed by linear regression analysis. The resulting equation ($R^2(\text{adj}) = 0.980$; $\text{STD} = 0.012$), expressed below (eq 4), proves the relationship between these two kinds of information

**Figure 4.** Evolution of elaidic acid concentration versus heating time (A) and the area under the band at 967 cm^{-1} ($978\text{--}960 \text{ cm}^{-1}$) (B).

(**Figure 4B**) and confirms the chemical assignment attributed to this band.

$$\text{peak area } 978\text{--}960 \text{ cm}^{-1} = 0.035 + 0.126 \times [\% \text{C18:1}t] \quad (4)$$

The fatty acid composition of the samples (**Table 2**) also points out that the major contributor to the selected IR band is the elaidic acid. The other forms of isolated trans double bonds are less significant. Likewise, the fatty acid analysis verified the relatively low significance of conjugated trans fatty acid (C18:2*t*, C18:3*t*) in comparison with elaidic acid.

CONCLUSIONS

Although the increase of the trans region in the FTIR spectra of frying oils had been already reported, no study had been carried out to study the applicability of this change in frying monitoring. Olive oil, as a model frying oil, offers two advantages in this regard. First, the low levels of linoleic and linolenic acids as compared with that of oleic acid allows us to dismiss the IR band assigned to conjugated trans/cis double bonds and track the trans content levels by measuring the area of a single band ($978\text{--}960 \text{ cm}^{-1}$). Second, the legal limits of elaidic acid for virgin olive oil— $\leq 0.05\%$ in virgin olive oil and 0.20% in olive oil (37)—ensure the basal levels of this area and allow for building a model that consistently works as long as virgin olive oil is used for frying. As FTIR is a secondary analytical method that in most of cases requires reference data, the increase of the IR trans band was tested by fatty acid analysis that proved, in addition, the relationship between trans content and %TPC. The levels of elaidic acid (around 2%) determined in the samples were above the quantification limit by ATR-FTIR (38). As an additional benefit, the measurement of the IR trans band regressed against %TPC provides information on trans content, which is of major concern and have nutritional and health implications. The increase of trans fatty acids during frying is reflected in the final fried food, which may contain similar concentrations of trans species, and therefore it cannot boast a zero trans claim (39). Better results are expected with continuous and industrial frying, where all the parameters are controlled and the periods with no frying operation are removed.

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