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Food Chemistry 77 (2002) 503–510

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils

María D. Guillén*, Nerea Cabo

Tecnología de los Alimentos, Facultad de Farmacia, Universidad del País Vasco, Paseo de la Universidad 7, 01006 Vitoria, Spain

Received 22 May 2001; received in revised form 14 September 2001; accepted 14 September 2001

Abstract

Different edible oils, including safflower, sunflower, rapeseed and olive oils, were submitted to oxidation in a convection oven with air circulating at 70 °C. Their oxidation process was monitored by Fourier transform infrared spectroscopy by periodically collecting duplicate spectra from films of pure oil between two KBr disks. The frequency and absorbance of each infrared band were automatically registered by a macro program. Classic chemical methods for determining primary and secondary oxidation products, such as peroxide value and anisidine value respectively, were determined periodically. Changes observed in infrared data are useful indicators of edible oils oxidative stability and are closely related to changes observed in peroxide and anisidine values in the course of the oxidation of the samples. Fourier transform infrared spectroscopy may be able to substitute classic oxidation indices in the determination of oxidative stability or antioxidant activity due to its simplicity, low cost and time saving.

Keywords: Edible oils; Oxidation; Oxidative stability; Fourier transform infrared spectroscopy; Peroxide value; Anisidine value

1. Introduction

The oxidative stability of oils could be defined as their resistance to oxidation. This is an important indicator of performance and shelf-life, and depends on the composition of the sample and on the conditions to which it is subjected. Any study of the oxidative stability of oils require the establishment of the conditions under which the sample is oxidised, knowledge of the oxidation process, and a method to determine the rate at which this process takes place; the same requirements should be taken into account in studies of antioxidant activity.

Oxidative conditions can include a determined flow of air or of oxygen, the heating of the sample at a determined constant temperature, the presence or absence of light and catalizers, the exposure of the sample to a determined light frequency, etc. Some of these oxidative conditions are used in the well-known accelerated tests of oxidative stability (Frankel, 1993). However, it must be taken into account that the oxidative conditions can influence the mechanism of the degradation process.

Although other degradation mechanisms are also possible, the oil degradation process has been generally established as being a free radical mechanism yielding hydroperoxides, also called primary oxidation products, which in their turn degrade into aldehydes, ketones, lactones, alcohols, acids, etc., or secondary oxidation products.

The methods used to determine the rate at which the oxidation process advances are related to the measurement of the concentration of primary or of secondary oxidation products or of both, or to the amount of oxygen consumed during the process. Among those based on the evolution of the concentration of primary oxidation products, peroxide value (PV), which measures hydroperoxide concentration, is one of the most widely used. Different approaches have been proposed for its determination, most of them based on chemical reactions (Gray, 1978).

Hydroperoxides show diene or triene conjugated double bonds coming from 1,4-pentadiene or from 1,4,7-octatriene units present in linoleic or in linolenic acyl groups respectively. The measurement of the absorbance of conjugated dienes at 232–234 nm, and of conjugated trienes at 268 nm has also been used in

* Corresponding author. Fax: +34-945-130756.
E-mail address: knpgulod@vf.ehu.es (M.D. Guillén).

0308-8146/02/$ - see front matter
PII: S0308-8146(01)00371-5

studies of oxidative stability; however the values obtained with this method are only valid for comparison if the proportion of 1,4-pentadiene or of 1,4,7-octatriene units in the oil samples subject of study are of the same order. In addition, secondary oxidation products such as some ethylenic diketones and conjugated ketodienes and dienals also absorb at 268 nm, interfering with the measurement of primary oxidation products containing conjugated trienes (Warner & Eskin, 1995).

Some other methods, are based on the concentration of secondary oxidation products including aldehydes, ketones, acids, alcohols, lactones, ethers, hydrocarbons and furan derivatives. The total content of carbonyls (aldehydes and ketones), also called carbonyl value, has been used in different oxidative stability studies, with various approaches to its determination, most of them based on carbonyl groups chemical reactions; nevertheless, these have been criticised because the determination conditions cause the degradation of hydroperoxides into carbonyl derivatives, giving erroneous results (Gray, 1978). Likewise the determination of the content, in the oil or in its headspace, of concrete aldehydes has also been used for measuring oxidative stability. Determination of α and β-alkenals content, basis of the anisidine value (AV) method, determination of malonaldehyde content, basis of the so called thiobarbituric acid test (or TBA), and determination of hexanal content in the headspace of the oil sample, are three examples of these latter methods. In the same way, the content of other secondary oxidation products such as low molecular weight acids is the basis of the Rancimat equipment used to measure oxidative stability.

The above methods measure the oxidative stability with different degrees of approximation depending on the error sources of each method, and their advantages and disadvantages have been extensively described (Warner & Eskin, 1995). It should be noted that each of the above methods can only afford information on the concentration of the corresponding compound or group of compounds at different moments during the process.

Mid Fourier transform infrared (FTIR) spectroscopy gives information about the different functional groups present in a sample, not only about a kind of compounds as do the above methods. As a result one might think this technique useful in order to study the oxidation process of edible oils. In previous papers we have studied the changes undergone in the frequency (Guillén & Cabo, 1999b) and in the absorbance (Guillén & Cabo, 2000) of different bands of the FTIR spectra of several edible oils throughout their oxidation process and it was concluded that this technique could be of use in studying oxidative stability, although no relationships between infrared data and any of the above-mentioned methods were tested. The aim of this paper is to study to what degree data, afforded by infrared spectroscopy in a simple way, are as valid for measuring the oxidative stability of edible oils as some of the classic indices mentioned above. Although some authors have reported different methods to determine PV (Van de Voort, Ismail, Sedman, Dubois, & Nicodemo, 1994) and AV (Dubois, Van de Voort, Sedman, Ismail, & Ramaswamy, 1996) of edible oils from FTIR spectra using standard calibration mixtures and the Partial Least Squares method, this is not the aim of this paper. The purpose of this paper is to establish if changes observed in the infrared spectra are simultaneous with changes observed in PV and AV indices, and to see to what extent infrared data can be used instead of them, taking into account that characteristics such as simplicity, low cost and time saving are important in a method.

2. Materials and methods

2.1. Sample collection

The samples subject of study were five edible oils acquired from local supermarkets, with very different proportions of oleic, linoleic and linolenic acyl groups, namely olive oil made of a mixture of refined and virgin olive oil, two different samples of refined sunflower oil, named A and B, safflower oil, and rapeseed oil.

2.2. Sample oxidation

Ten grams of sample were weighed in polystyrene Petri dishes of 80 mm diameter and 15 mm high and placed in a *Selecta* convection oven, whose temperature was maintained at 70 °C with a stability of ±0.5%. The Petri dishes were introduced into the oven without their lids to facilitate the exposure of the sample to the circulating air. The oxidation of each sample was carried out in duplicate, for this reason, two Petri dishes of each oil sample were prepared per day. The position of the Petri dishes inside the oven should be in a zone where the heat and air flow will be constant throughout the oxidation experiment. The determination of the PV, AV and the acquisition of the infrared spectra were carried out periodically.

2.3. FTIR spectra

The infrared spectra were recorded on a FTIR spectrometer Bruker Vector 33 (Bruker Optic Gmbh), and on a FTIR spectrometer Nicolet Magna-IR 550 (Nicolet Instrument, Madison WI), interfaced to a personal computer, operating under Opus NT software (version 2.0) and under Nicolet Omnic software (version 3.1) respectively.

A film of a small amount of each sample, approximately 2 μl, was deposited between two disks of KBr,

avoiding the presence of air, as in previous studies (Guillén & Cabo, 1997, 1998, 1999a, 1999b, 2000). Duplicate spectra were collected from each sample at daily intervals, until the samples became so viscous that it was impossible to deposit a film of the sample between the KBr disks. All spectra were recorded from 4000 to 500 cm^{-1}, co-adding 32 interferograms, with a measurement accuracy in the frequency data at each measured point of 0.01 cm^{-1}, due to the laser internal reference of the instrument. If the measured point agrees with the maximum absorbance of the band, the accuracy of the frequency data of the band is 0.01 cm^{-1}, if not, the frequency measures are not so accurate. In order to avoid high noise levels the spectra were collected with a resolution of 4 cm^{-1} to give, after Fourier transformation and zero-filling, a data point spacing of approximately 1.9 cm^{-1}. Recorded spectra with higher resolution give similar frequency data for all samples but with higher noise level and their registration takes more time.

The frequency of each band was obtained automatically by using the "find peaks" command of the instrument software. This procedure avoids the experimental errors associated with the subjectivity of the external operators. The assignment of the bands to a concrete functional group vibration mode was made from comparison with spectral data literature as well as with reference compounds spectra included in the software spectral library.

The height and the area of each band were measured in absorbance automatically by using the equipments software taking two baselines: 3750–2472 and 1900–530 cm^{-1}. This procedure avoids the experimental errors associated with the subjectivity of the external operators.

2.4. PV and AV determination

PV was determined by the standard 965.33 AOAC iodometric method (AOAC, 1990). AV was determined by the standard 2504 IUPAC method (IUPAC, 1987) using an Unicam UV-Vis spectrophotometer. The data are presented as mean ± standard deviation of duplicate determinations and are representative of two oxidation experiments.

3. Results and discussion

The oil samples were submitted to oxidative conditions under a constant air flow, in darkness at 70 °C. Periodically, their PV and AV were determined and their FTIR spectra were recorded. Fig. 1 shows the PV, given in O_2 mequiv/kg, on several days of their oxidation process. Problems in relation to the accuracy of PV determination have been widely commented on by several authors (Gray, 1978; Warner & Eskin, 1995). The standard deviations of PV determinations are small when the samples have low hydroperoxide concentrations, and large when these are high; in this latter case, variation coefficients near 10% have been found in some determinations. Similar variation coefficients and deviations have been found by other authors (Van de Voort et al., 1994). However, it has been observed that hydroperoxide generation and degradation rates have more influence on the accuracy of PV determination than the amount of hydroperoxides present in the sample. High hydroperoxide generation and degradation rates made the obtention of reproducible PV values difficult.

In the non-oxidised oil samples, the highest hydroperoxide concentration was found in safflower oil (10.9 ± 0.0) whereas olive and rapeseed oils show similar concentrations (8.0 ± 1.0 and 9.0 ± 0.0, respectively) and the lowest hydroperoxide concentrations were in sunflower oils (sunflower A 3.7 ± 0.2 and sunflower B 2.9 ± 0.0). However these initial PV had no relationship to the rate at which these oil samples were degraded.

Although the hydroperoxide generation and degradation rates were different in each sample, there were some general features. In all samples PV increased from the beginning of the oxidation experiment to reach a maximum value, after which a more or less pronounced decreasing of PV was observed. In the olive oil samples, as Fig. 1 shows, different stages are observed from the beginning of the oxidation experiment until PV reaches the maximum value. During the first 11 days PV increases very slowly, close to 2 O_2 mequiv/kg day; from day 11 to 13 a pronounced increase in PV is observed; from day 13 to day 16 the increase of PV continues but is not as pronounced as before. In the other oil samples the increase in PV is very fast and for this reason the differentiation between stages is not so clear as in the

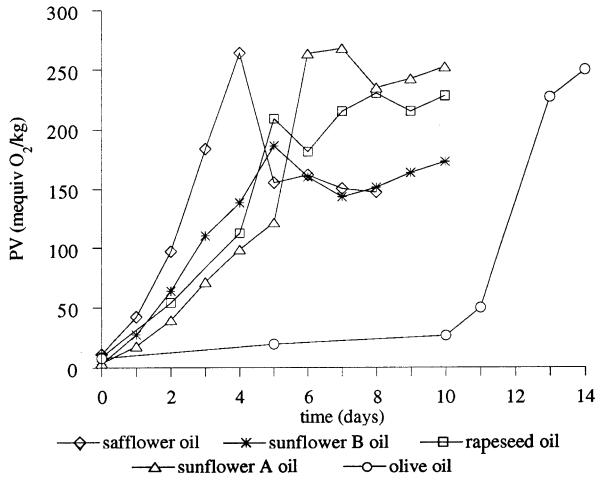

Fig. 1. PV values of the oil samples versus the time in days of their oxidation process.

olive oil sample. In sunflower A the pronounced rise in PV is produced between days 5 and 6, in rapeseed and in sunflower B between days 4 and 5 and in safflower between days 3 and 4.

AV represents the content of secondary oxidation products such as α and β-alkenals and of all those compounds able to react with *p*-anisidine reagent. Duplicated AV determinations were made periodically during the course of the oxidation of safflower, sunflower B, rapeseed and olive oil samples. In non-oxidised oil samples the highest AV is in sunflower oil (7.3 ± 0.4); rapeseed (3.5 ± 0.1) and olive (5.1 ± 0.3) oils show intermediate values, and the lowest AV is in safflower (1.1 ± 0.1) oil sample. Some authors have indicated that these values are comparable only within each oil type because initial AV varies among oil sources (Warner & Eskin, 1995). Fig. 2 shows that the rate of AV increase follows a general pattern in all oil samples throughout the oxidation process. They all have a first stage in which AV increase rate varies from an almost nil value, as in the case of sunflower and safflower oil samples, to very small values in olive oil sample, or to significant values in rapeseed oil sample. After this there is a second stage in which AV increase rate is great in all samples, especially in the rapeseed oil sample. From comparison of Figs. 1 and 2 it can be observed that a high rate of hydroperoxide generation (Fig. 1) does not always involve a high rate of generation of secondary oxidation products (Fig. 2). In addition, in some oil samples such as olive and rapeseed oil, the generation of secondary oxidation products begins almost simultaneously with the generation of hydroperoxides, and in others such as sunflower and safflower oil the degradation of hydroperoxides begins when the concentration of these compounds is appreciable.

In spite of these facts the AV increase rate coincides in time with the change of the PV increase. That is to say, throughout the oxidation process there is a period of time after which a significant increase in the generation of primary and secondary oxidation products is produced and this is detected by both PV and AV.

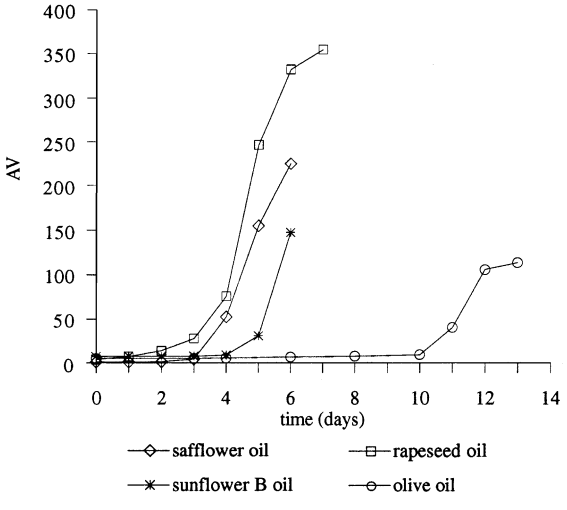

Fig. 2. AV determinations of the oil samples versus the time in days.

The FTIR spectra afford information on the functional groups of the sample. In previous papers the assignment of the FTIR spectrum bands of edible oils and their changes throughout the oxidation process have been commented on (Guillén & Cabo, 1999b, 2000). Fig. 3 shows the infrared spectrum of the sunflower A oil. It is well known that compounds present in a mixture in very low proportions give very weak bands which are not detectable in the infrared spectrum. As a result the band of hydroperoxide functional group near 3444 cm^{-1} is not observed in the infrared spectrum of non-oxidised sunflower oil. This is also true for the other oil samples studied.

In this region there is, in all the non-oxidised oil infrared spectra, a band near 3470 cm^{-1} associated with the overtone of the glyceride ester carbonyl absorption. As the oxidation process advances the concentration of hydroperoxide groups in the sample increases and also its absorption in the infrared spectrum. This functional group gives a broad band, so it overlaps with that of the overtone of the glyceride ester groups, producing a decreasing in the frequency value of the glyceride original band together with an increasing in its absorbance. For this reason, frequency and absorbance of this band can give information about the hydroperoxide generation throughout the oxidation process.

Fig. 4 shows the evolution of this band in the infrared spectrum of sunflower A oil during the oxidation process. It can be observed that in the first days of the process there are no apparent changes in the band (Fig. 4, days 0–5), but its frequency value diminishes slowly due to the generation of hydroperoxides; as the process advances, hydroperoxide concentration increases, and this band suffers a deformation (Fig. 4, day 6). Afterwards sharp and visible changes are observed, which include shifting of the maximum frequency of the band towards smaller values, broadening of the band and rising of its intensity due to the presence of a significant proportion of hydroperoxides in the sample (Fig. 4, days 7–8). In advanced stages of oxidation the frequency of this band suffer a new shifting towards values near to those of the original band in non-oxidised oil samples (Fig. 4, days 9–14); this fact is probably due to the decreasing of hydroperoxide concentration and the appearance of new bands, at approximately 3530 cm^{-1}, due to alcohols, secondary oxidation products, which overlap with that of the hydroperoxide groups. This is also true for the other oil samples studied.

Fig. 5 shows the variation of the frequency in band near 3470 cm^{-1} throughout the oxidation process in the five oil samples. In all cases there is a period of time in which the frequency of this band only suffers a slight decrease; this has been previously called first stage (FS;

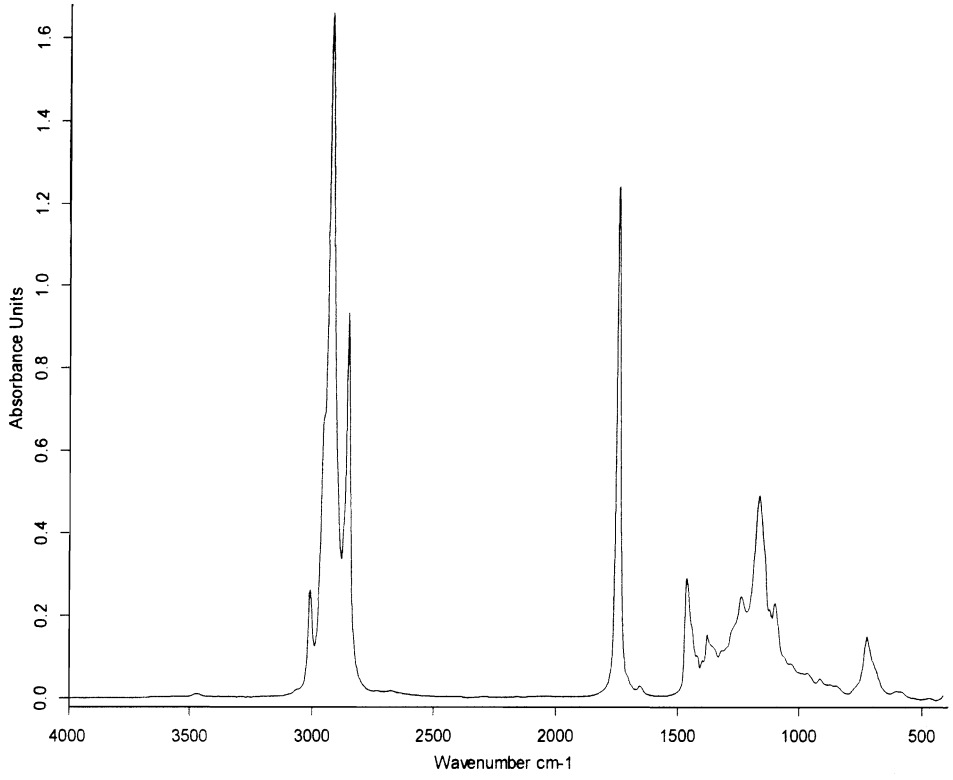

Fig. 3. FTIR spectra of the sunflower A oil sample.

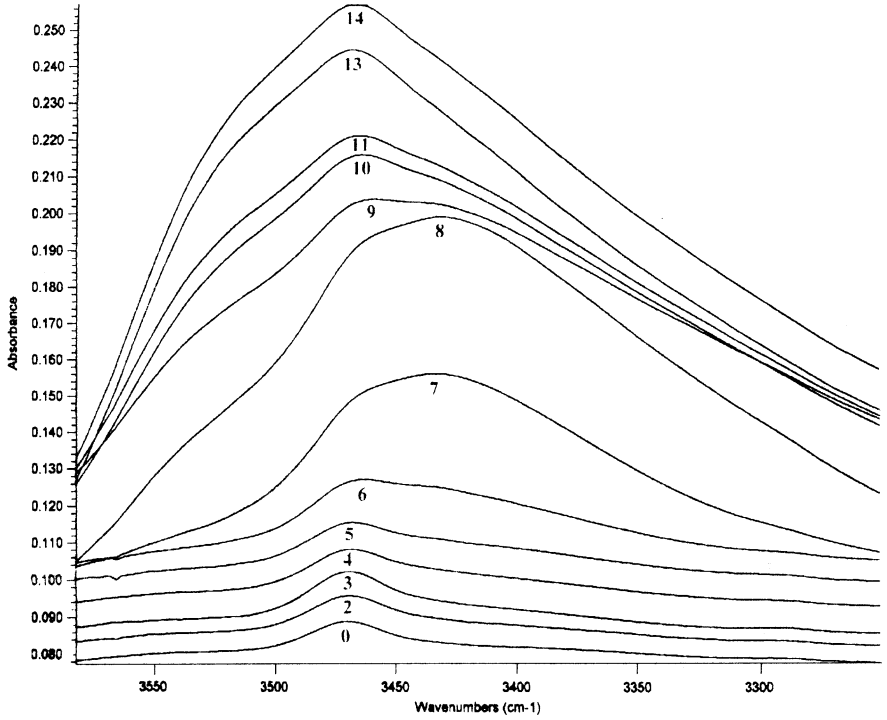

Fig. 4. Changes produced in the region between 3600 and 3250 cm^{-1} of the infrared spectrum of sunflower A oil on different days of the oxidation process.

Guillén & Cabo, 1999b). At the end of this period a deformation of this band is clearly perceptible with the naked eye. Afterwards there is a sharp decreasing in the frequency of the band to reach values near to 3444 cm^{-1}, associated with hydroperoxide groups, and so begins the second stage (SS) after which there is a new shifting of the frequency of the band to values close to the original one (TS). The beginning of this second stage is coincident with maximum hydroperoxide concentrations in the samples. Consequently, either the duration of the FS, at the end of which the deformation of the band is evident, or the time when the sharp change in the frequency of this band is produced, that is the transition time between FS and SS or the beginning of SS, can be taken as a measure of the oxidative stability. Oxidative stability expressed as the duration of FS is 3 days for safflower oil, 4 days for sunflower B and rapeseed oils, 6 days for sunflower A oil, and 12 days for olive oil. These period of time are in fairly good agreement with those in which the most pronounced increase in PV and AV is produced.

Relationships between PV, in the concentration range in which they can be determined with a certain degree of accuracy, and frequency values of the above-cited band have been found. Both set of data fit linear equations with high correlation coefficients, as Table 1 shows. From these results it can be concluded that frequency values of band near 3470 cm^{-1} may predict hydroperoxide concentrations in these ranges and are of great interest to determine oxidative stability. Frequency data are usually given in FTIR spectrometers with an accuracy of 0.01 cm^{-1} and measurement only takes the couple of minutes needed to put a film of the oil sample between two KBr disks and register the spectrum. The frequency values of all bands of the spectrum are given by the equipment automatically. For these reasons the possibilities of error are very small. In the same way, there are also close relationships between AV and frequency data of the band near 3470 cm^{-1}. Table 2 gives the coefficients of the equations together with the correlation coefficients that relate AV with frequency data.

In the infrared spectrum near 3006 cm^{-1} appears the band of *cis* double bonds CH groups (Fig. 3). In previous papers on non-oxidised oil samples it has been shown that the frequency of this band is related to the composition of the oil; oils with a large proportion of polyunsaturated acyl groups have higher frequency values than those with a large proportion of monounsaturated or saturated acyl groups (Guillén & Cabo, 1997). During the oxidation process, the disappearance of *cis* double bonds is produced, as well as isomerization of *cis* to *trans* groups, alongside with hydroperoxide generation. For this reason frequency and absorbance of band near 3006 cm^{-1} suffer changes as the oxidation process advances. The frequency of this band remains practically constant during FS, after which it decreases. Fig. 6 shows the evolution of the frequency of this band throughout the oxidation process. It can be observed that the time at which the frequency of this band begins to decrease is also a measurement of the oil stability, similar to PV or AV. There are other bands, such as those in Fig. 3 near 1238, 1163 and 1118 cm^{-1}, respectively, related to the stretching vibrations of the C–O

Table 1
Coefficients of the equations, $F_{3470} = a + b\mathrm{PV}$, where F_{3470} is the exact frequency of the maximum of absorbance of the band near 3470 cm^{-1}, together with the interval of days used in the adjustment and the correlation coefficients

Equation no.	Oil sample	Day interval	*a*	*b*	*R*
1	Safflower	0–3	3471.27	−0.02	−0.9806
2	Sunflower, B	0–4	3470.68	−0.01	−0.9945
3	Rapeseed	0–4	3469.21	−0.01	−0.9584
4	Sunflower, A	0–6	3469.09	−0.01	−0.9976
5	Olive	0–13	3473.16	−0.04	−0.9911

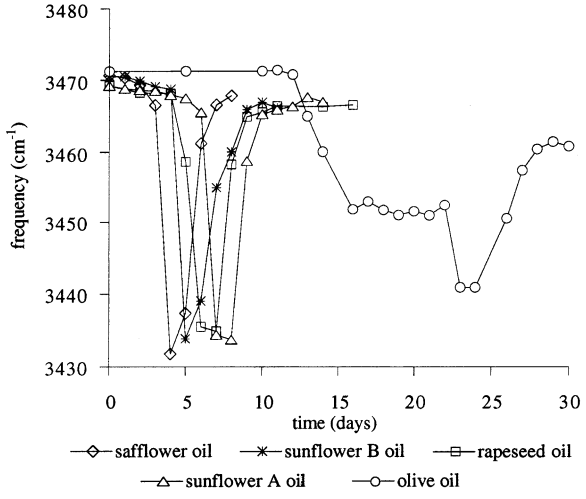

Fig. 5. Frequency values of band near 3470 cm^{-1} versus time for the oil samples under oxidative conditions.

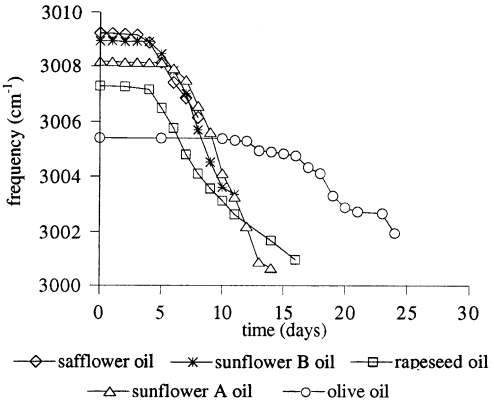

Fig. 6. Frequency values of band near 3006 cm^{-1} versus time for the oil samples under oxidative conditions.

groups of esters, whose frequency values show an evolution similar to that observed in band near 3006 cm^{-1}, throughout the oxidation process.

The non-oxidised oil samples show the band of the triglyceride ester groups at 1746 cm^{-1} (Fig. 3). During the oxidation process hydroperoxides degrade into secondary oxidation products such as aldehydes and ketones, which give bands near 1728 cm^{-1}. These bands overlap with that of ester group at 1746 cm^{-1} causing a broadening of the band and a decreasing of its frequency. Evolution of the frequency of this band throughout the oxidation process is given in Fig. 7.

Changes observed in the frequency of all above mentioned infrared bands match with changes in their absorbances. In previous papers it has been proved that changes in absorbance of the different bands of the FTIR spectra throughout the oxidation process of edible oils leads to the same conclusions as changes in the frequency of the same bands (Guillén & Cabo, 2000). For this reason it could be assumed that all above correlations between frequency data and classic PV and AV determinations are also true for absorbance of the bands.

It is evident that FTIR spectroscopy is a technique affording a great deal of information about oil samples in a simple way with a great saving in time and cost. Although PV, AV and other classic parameters are used in the measurement of oil oxidative stability, infrared data can be used for the same task with many advantages: the infrared spectrum can give information on the different functional groups present in the sample in significant proportions; the amount of oil sample needed to collect a spectrum is very small; preparation of the sample and collection of the spectrum only takes a couple of minutes; error sources in the sample preparation, spectrum collection, and frequency and absorbance determination are minimal because these latter are automatic and because the sample preparation does not require any manipulation; finally, the cost is also minimal because no reactive is needed. For these reasons infrared data may replace the classic parameters not only in routine controls but also in research on oxidative stability or on antioxidant activity.

Table 2
Coefficients of the equations, $F_{3470} = a + b \times AV$, where F_{3470} is the exact frequency of the maximum of absorbance of the band near 3470 cm^{-1}, together with the interval of days used in the adjustment and the correlation coefficients

Equation no.	Oil sample	Day interval	a	b	R
6	Safflower	0–4	3471.04	−0.56	−0.9979
7	Sunflower, B	0–6	3471.67	−0.24	−0.9836
8	Rapeseed	0–6	3471.86	−0.07	−0.9986
9	Olive	0–15	3473.72	−0.22	−0.9929

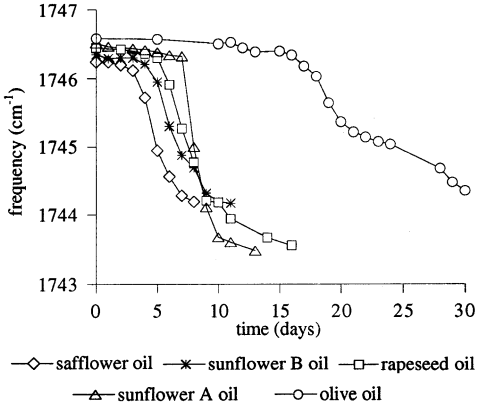

Fig. 7. Frequency values of band near 1746 cm^{-1} versus time for the oil samples under oxidative conditions.

Acknowledgements

This work has been supported by the Dirección General de Investigación del Ministerio de Ciencia y Tecnología (Proyecto AGL 2000-1696) and the Universidad del País Vasco (Proyecto UPV 101.123-EB087/99).

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