Usefulness of the Frequency Data of the Fourier Transform Infrared Spectra To Evaluate the Degree of Oxidation of Edible Oils

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The oxidation process of 13 edible oil samples with different proportions of oleic, linoleic, and linolenic acyl groups has been studied using Fourier transform infrared spectroscopy. The oxidation experiments were carried out by heating the samples in a convection oven at 70 °C. Duplicate spectra were recorded from a film of pure oil between two disks of KBr for each sample every day during the course of the oxidation, and frequency data of each band of the spectrum were collected automatically. Changes in the values of the frequency of most of the bands of the spectra were observed. The shiftings of the frequency value of specific bands allowed one to distinguish between the different stages of the oxidation process and to establish the oxidation degree of each oil sample. This methodology could be useful to evaluate the oxidative stability of edible oils in a simple, fast, and accurate way.

Keywords: *Edible oils; oxidation; Fourier transform infrared spectroscopy; frequency shiftings; oxidation degree; oxidative stability*

INTRODUCTION

Infrared spectroscopy allows the qualitative determination of organic compounds because the characteristic vibrational mode of each molecular group causes the appearance of bands in the infrared spectrum at a specific frequency, which is further influenced by the surrounding functional groups. It is also possible to carry out quantitative analyses, as the intensity of the bands is proportional to the concentration of the functional group, according to Lambert−Beer's law. In the past, due to the major difficulty for the dispersive instruments to obtain fast reproducible results, infrared spectroscopy has scarcely been used in food studies. However, with the incorporation of the Fourier transformation, the application of this technique has increased in all fields (Guillén et al., 1992, 1995), including foods (Guillén and Manzanos, 1996; Guillén and Cabo, 1997a,b). One of the advantages of the Fourier transform technique is the high accuracy and reproducibility in the measurement of the frequency values.

Mid-infrared spectra have been used to characterize edible oils and fats (Bartlet and Mahon, 1958; Ahmed et al., 1986; Guillén and Cabo, 1997b) because they show differences in the intensity and the exact frequency at which the maximum absorbance of the bands appears, according to the nature and composition of the sample under study. With the utilization of multivariate statistical methods such as principal component analysis (Safar et al., 1994; Dupuy et al., 1996) or discriminant analysis (Lai et al., 1994), the differences between spectra have been emphasized and the classification of different commercial edible oils and fats has been carried out. Recently, Guillén and Cabo (1997a) have observed close relationships between the frequency of some bands of the fingerprint region and the proportion of mono- and polyunsaturated and saturated acyl groups in 13 samples of several edible oils and lard, which may be useful for predictive purposes. Infrared spectroscopy has also been widely applied to the determination of trans unsaturation by means of the quantification of the intensity of the band at 967 cm^{-1} assigned to isolated trans double bonds vibrational mode, and the method has been standardized by IUPAC (1987), AOCS (1989), and AOAC (1990). In addition, some methods have been developed from the Fourier transform infrared (FTIR) spectra data for the fast accurate determination of some classical indices of edible oils and fats such as iodine value and saponification number (Van de Voort et al., 1992), free fatty acids (Ismail et al., 1993), cis and trans content (Van de Voort et al., 1995), and solid fat content (Van de Voort et al., 1996).

Besides characterization, other major concerns from the production quality standpoint are the determination of the oxidation level of fatty products, due to economic and safety considerations, as well as the knowledge of the oxidative stability of fatty foods. The experimental techniques for the measurement of lipid oxidation have been the subject of several reviews (Gray, 1978; Rossell, 1986, 1989; Hamilton, 1989; Warner and Eskin, 1995); although infrared spectroscopy is included in some of these revisions (Gray, 1978; Rossel, 1989), until recently it has hardly received attention.

Traditional methods for establishing the oxidative state of edible oils and fats are chemical methods based on the measurement of the concentration of the main products generated in the process. That is true for peroxide value, the most commonly used, which is based on the chemical determination of hydroperoxide concentration and which can be considered as an indicator of the initial stages of oxidation but cannot be indicative of the current extent of oil or fat oxidation because hydroperoxides decompose rapidly; nor is peroxide value useful for monitoring the degradation of oils without

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Table 1. Percentage by Weight of Polyunsaturated (%P), Saturated (%S), and Monounsaturated (%M) Acyl Groups of the Samples

	A,[a] virgin olive oil	E,[b] olive oil	F,[a] sesame oil	G,[b] sunflower oil	I,[a] sunflower oil	J,[b] corn oil	M,[a] seed oil	P,[a] soybean oil	R,[b] safflower oil	T,[a] seed oil	U,[b] rapeseed oil	V,[b] peanut oil	W,[b] walnut oil
%P	12	8	38	60	59	57	56	64	75	64	30	20	71
%S	13	13	13	13	13	13	13	12	10	13	8	17	9
%M	75	79	49	27	27	30	31	24	15	23	62	63	16

[a] Determined following the methodology described by Guillén and Cabo (1997a). [b] Determined following AOAC Method 963.22 (AOAC, 1990).

hydroperoxide formation as intermediate compounds. Infrared spectroscopy has been used to establish methods with high reproducibility for peroxide value determination, after previous calibration with standards (Van de Voort et al., 1994a; Ma et al., 1997).

Other chemical methods to establish the oxidation degree in edible oils are based on the determination of secondary oxidation products such as anisidine value, which basically measures the concentration of aldehydes, considered mainly responsible for the off-flavors in oxidized oils and fats. A method to determine the anisidine value by means of infrared spectroscopic data and calibrations with standards has also been published (Dubois et al., 1996).

The usefulness of infrared spectroscopy, together with other techniques, for characterizing pure compounds formed by oxidation of fatty acids and esters is well-known (Chan and Levet, 1977a,b; Frankel et al., 1977, 1982; Neff et al., 1978, 1982; Thomas and Prior, 1980; Wu et al., 1992). More recently, Van de Voort et al. (1994b) have studied the oxidation process of cottonseed and safflower oils, carried out on a heated horizontal ATR crystal or heated in a heating mantle, by measuring band height at preselected frequencies in FTIR spectra; using standards, these authors have proposed a quantitative approach, based on the measurement of the absorbance of specific bands, for determining the oxidative state of an oil, defined in terms of the percentage of hydroperoxides, alcohols, and the total carbonyl content.

In this paper the study of the oxidation process of 13 edible oil samples using FTIR spectra has been carried out. The oxidation experiments were carried out in all cases by heating the samples in a convection oven at 70 °C until the sample acquired a high viscosity. The samples studied included oils with very different proportions of oleic, linoleic, and linolenic acyl groups. The aim of this study was to explore whether the oxidation process follows the same patterns in all samples studied and if infrared spectroscopy is useful not only to provide information of the oxidation degree of an oil sample but also to measure, in a simple, fast, and accurate way, the quality of an oil by its oxidative stability. The usefulness of the frequency values of the different bands of the infrared spectra for the purpose mentioned is tested.

EXPERIMENTAL PROCEDURES

Sample Collection. A broad collection of 13 edible oils was acquired from local supermarkets or from producers, in order to have samples with very different proportions in oleic, linoleic, and linolenic acyl groups. The sample collection includes one sample of extra virgin olive oil, designated A; one sample of olive oil made of a mixture of refined and virgin olive oil, named E; one sample of virgin sesame oil, F; two samples of refined sunflower oil, G and I; one sample of refined corn seed oil, J; two samples of seed oil of unknown vegetable origin, designated M and T; one sample of refined soybean oil, P; one sample of safflower oil, R; one sample of rapeseed oil, U; one sample of African peanut oil, V; and finally one sample of walnut oil, W.

Sample Oxidation. Ten grams of each sample was weighed in polystyrene Petri dishes of 80 mm diameter and 15 mm high and placed in a Selecta convection oven, the temperature of which 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 or triplicate.

FTIR Spectra. The infrared spectra were recorded on an FTIR spectrometer, Nicolet Magna-IR spectrometer 550 (Nicolet Instrument Corp., Madison WI), interfaced to a 486 personal computer operating under Windows-based Nicolet Omnic software (version 3.1). The instrument was purged with a Balston dryer (Balston, Lexington, MA) to minimize water vapor and CO_2 interferences.

Spectral Acquisition. A film of a small amount of each sample (~2 μL) was deposited between two disks of KBr, avoiding the presence of air, as in previous studies (Guillén and Manzanos, 1996; Guillén and Cabo, 1997a, 1998). Duplicate spectra were collected from each sample, every day during the course of the oxidation process, 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 He−Ne 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. 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 ~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.

Study of the Spectra. The frequency of each band was obtained automatically by using the "find peaks" command of the instrument software with an adequate threshold value near 82%. This procedure avoids the experimental errors associated with the subjectivity of the external operators. The assignation of the bands to a specific functional group vibration mode was made from comparison with spectral data literature as well as with reference compound spectra included in the software spectral library.

RESULTS AND DISCUSSION

The samples were kept under oxidative conditions, at 70 °C in a convection oven, and an infrared spectrum of each one was recorded daily to detect the changes.

The infrared spectra of the samples in their original state are similar; however, some differences have been found, and they are related to the frequency and intensity of some bands, as well as to the presence or absence of others (Guillén and Cabo, 1997a, 1998). These differences in the infrared spectra are due to the different composition of each sample. In Table 1 are

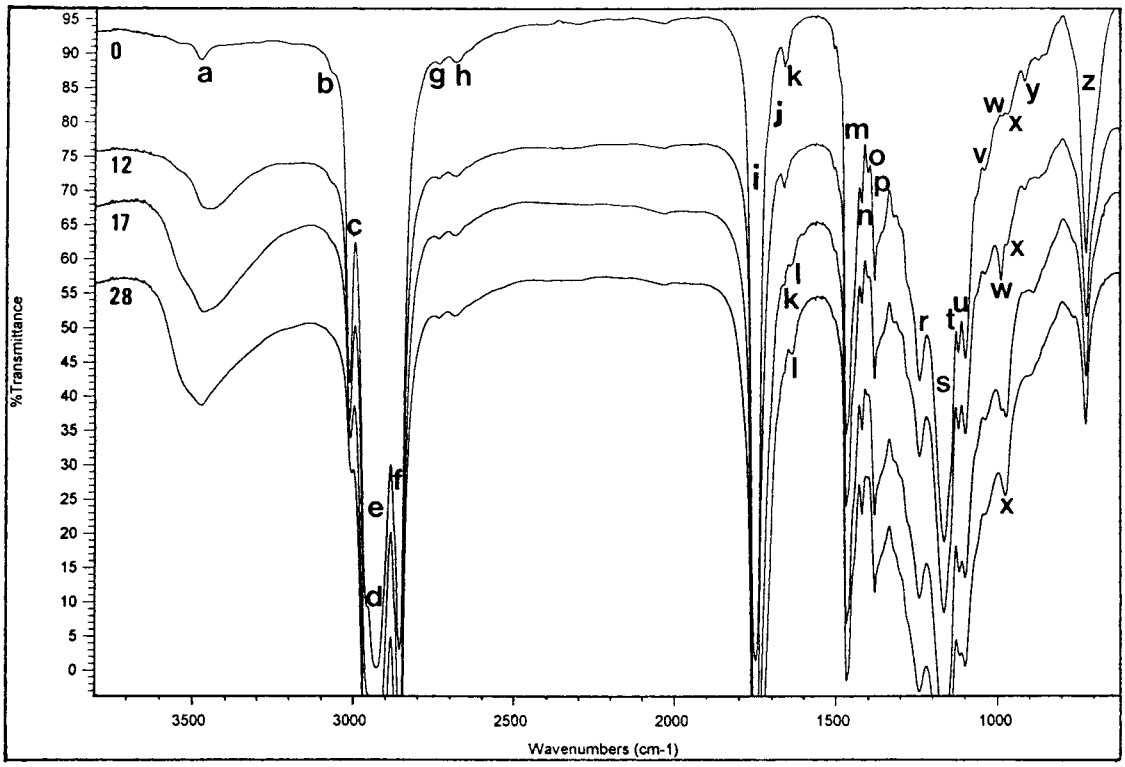

Figure 1. FTIR spectra of the sesame oil sample (F) after 0, 12, 17, and 28 days respectively, under oxidative conditions. The infrared bands are indicated with letters (from **a** to **z**) and are referred to in this way throughout the text.

Table 2. Different Stages in Days Observed in the Oxidation Process of 13 Oil Samples

	A, virgin olive oil	E, olive oil	F, sesame oil	G, sunflower oil	I, sunflower oil	J, corn oil	M, seed oil	P, soybean oil	R, safflower oil	T, seed oil	U, rapeseed oil	V, peanut oil	W, walnut oil
FS	21	19	11	5	5	8	5	4	4	6	7	11	3
SS (d_i–d_f)[a]	22–48	20–40	12–16	6–10	6–10	9–13	6–9	5–8	5–6	7–9	8–12	12–17	4–6
TS (d_i–d_f)[a]	49–56	41–56	17–38	11–14	11–14	14–16	10–12	9–11	7–8	10–11	13–32	18–32	7–8
TT	56	56	38	14	14	16	12	11	8	11	31	31	8

[a] d_i = first day of this interval; d_f = last day of this interval.

given the proportions of saturated and mono- and polyunsaturated acyl groups of the different oils studied; it can be observed that a very broad range of proportions of the different acyl groups are among the samples. The tentative assignment of the majority of the infrared bands of different edible oil samples has been widely commented on in previous papers (Guillén and Cabo, 1997b, 1998).

The oxidation process of the different samples follows a general pattern. In Figure 1, as a descriptive example, four infrared spectra of the sesame oil (F), corresponding to different stages of the oxidation process, are included to distinguish with clarity the most important spectral changes produced during the oxidation of edible oils. Figure 1 shows the infrared spectra of the sesame oil sample at 0, 12, 17, and 28 days, respectively, under oxidative conditions. Changes similar to those observed in the infrared spectrum of the sesame oil have also been observed in the other samples; however, differences among samples are also observed and are basically due to the rate at which the changes are produced. In this study changes observed in the frequency values of the bands are taken into account.

Changes in the Spectral Region from 3700 to 3150 cm^{-1}. The spectral region between 3150 and 3700 cm^{-1} undergoes several changes during the oxidation process. Nonoxidized oils show a narrow weak band **a** between 3400 and 3500 cm^{-1}, having maximum absorbance frequencies between 3467 and 3470 cm^{-1} depending on the nature of the oil (see Figure 1). This band is usually assigned to the overtone of the glyceride ester carbonyl absorption. During several days in which the sample is under oxidative conditions, this spectral region remains unaltered; the duration of this period of time depends on the nature of the sample and can be called first stage (FS).

In Table 2 are given the FS values in days of different samples. It can be observed that oils with a high proportion of oleic acyl groups have larger FS periods than oils with a high proportion of linoleic and/or linolenic acyl groups, and among oils with similar proportions of oleic acyl groups and very different proportions of linolenic acyl groups, such as safflower versus walnut and peanut versus rapeseed, those oils richer in linolenic acyl groups have smaller FS values.

After this period, as the oxidation process advances, band **a** becomes wider, from approximately 3100 to 3600 cm^{-1}, and more intense; the frequency of the maximum absorbance is shifted to lower wavenumbers, the values depending on the sample. Frequency data of the maximum absorbance very close to 3448, 3437, or 3458 cm^{-1} have been observed in this interval for different samples. This shifting of the band is due to the overlapping between the original band and new absorptions caused

by hydroperoxides generated in the oxidation process, in agreement with other authors (Van der Voort et al., 1994b). The time interval in which the frequency of the maximum absorbance of band **a** begins to change from the original values and remains near 3448, 3437, or 3458 cm^{-1} can be called the second stage (SS) and represents the time interval in which hydroperoxides or primary oxidation products are being formed in appreciable proportions. Obviously, depending on the nature of the oil, different kinds of hydroperoxide isomers will predominate in the sample. So 8-, 9-, 10-, and 11-hydroperoxides have been described in the oxidation of methyl oleate (Neff et al., 1978); 9- and 13-hydroperoxides have been described in the oxidation of methyl linoleate (Chan and Levett, 1977a; Frankel et al., 1977); 9-, 12-, 13-, and 16- hydroperoxides have been described in the oxidation of methyl linolenate (Chan and Levett, 1977b); and the thermal oxidation of saturated fatty acids involves the formation of monoperoxides coming from the oxygen attack of all the methylene groups of the fatty acid (Crnjar et al., 1981). This stage could be considered to have ended when the concentration of hydroperoxides in the sample begins to decrease and agrees with the shifting of the frequency of the absorbance maximum of band **a** back to 3468 cm^{-1}.

In Table 2 are given the SS intervals for the different samples. It can be observed that the time interval in which the frequency of band **a** remains near the characteristic frequency of the hydroperoxide functional groups, which is when the proportion of hydroperoxides is appreciable, is small and very similar for all samples, varying from 2 to 5 days, except for olive and virgin olive oils, which are 20 and 26 days, respectively. It is noteworthy that the differences between samples are shown not only by the length of time of the FS but also of the SS.

More changes are observed in this region as the process advances; again, the so-called band **a** gets wider and the frequency of its maximum absorbance is shifted to higher values in all samples studied, near 3468 cm^{-1}. This latter shifting of the frequency of the maximum absorbance is due to the appearance of an increasing absorption near 3530 cm^{-1}, which overlaps with those previously cited and which produces an evident shoulder, and a decrease of the hydroperoxide groups absorption. The shoulder near 3530 cm^{-1} is produced by the presence in the sample of significant proportions of alcohols or secondary oxidation products, in agreement with other authors (Frankel et al., 1982; Van der Voort et al., 1994b). In the time interval from the shifting of the frequency, again near 3468 cm^{-1}, to the polymerization of the sample, secondary oxidation products are mainly generated, and the frequency of band **a** does not suffer more changes. This time interval can be called the third stage (TS).

Table 2 gives the TS interval values for different samples. It must be pointed out that the end of the oxidation process was considered to be when the viscosity of the sample was so high, due to its polymerization, that it was not possible to obtain a film to register the infrared spectrum. It can be observed that the duration of this interval is very small for oils rich in polyunsaturated acyl groups and that the higher values have been found in oils with significant proportions of oleic acyl groups; it must be pointed out that the total period of time (TT) from the beginning of the experiment to the polymerization of the oil sample is very closely related to the proportion of monounsaturated acyl groups (%M) in the original oil sample; in fact, there exists a high correlation coefficient between both sets of data given in Tables 1 and 2. The equation obtained that relates both sets of data is

$$\mathrm{TT\ (days)} = -5.81 + 0.73\%\mathrm{M}$$

with an R value of 0.9540 for the 13 oil samples studied here.

In Figure 2 are shown the changes produced in the region between 3580 and 3350 cm^{-1} of the infrared spectrum of sesame oil sample during the oxidation process. The sequences of the process described above can be observed in this example; among others, one of the most evident differences between samples is shown in the timing of each process stage, as can also be observed in Figure 3; in the latter, the frequency values versus time of the so-called band **a** are given for safflower (R), sesame (F), and olive (E) oil samples. It is evident not only that the degradation process in olive and virgin olive oil begins much later than in the other oil samples but also that the generation of primary and secondary oxidation compounds, such as hydroperoxides and alcohols, is much slower as FS, SS, and TS values show.

In conclusion, oil samples that have a broad band in this region are oils in which the oxidation process has begun. Values of frequency near 3445 cm^{-1} or lower indicate that the sample is in the SS stage in which the concentration of hydroperoxides is appreciable, and at the end of this stage the concentration of secondary oxidation products is also high. A broad band with frequency values near 3468 cm^{-1} indicates that the oil sample is in an advanced stage of oxidation and the concentration of the secondary oxidation products is appreciable. Obviously, the smaller the FS period is, the lower the oxidative stability of the oil sample will be and the faster the hydroperoxides will appear; in the same way, the smaller the SS period is, the higher the rate of formation of secondary oxidation products will be.

Changes in the Spectral Region between 3030 and 2600 cm^{-1}. The IR region between 3030 and 2600 cm^{-1} has bands due to different functional groups. In some oil samples a shoulder **b** at ~3025 cm^{-1} is present and is usually assigned to the stretching vibration of trans olefinic double bonds; however, the intensity of this shoulder does not increase as the oxidation process advances as could be expected, because it is known that during this process trans unsaturations are formed from cis double bonds. In fact, this shoulder is detectable during FS, SS, and almost all TS stages, but at the end of the latter this shoulder disappears.

The frequency of the cis double bond stretching vibration band **c** near 3006 cm^{-1} has been proved to be related to the oil composition. Oils with a high proportion of linolenic or linoleic acyl groups show higher frequency data for this band than oils with a high proportion of oleic acyl groups. For this reason the value of this frequency in nonoxidized oil samples varies from 3009 cm^{-1} in walnut oil to 3005 cm^{-1} for olive oil made of a mixture of virgin olive and refined olive oils. When the oil sample is maintained under oxidative conditions, there is a period of time in which the frequency of this band remains almost unaltered or suffers a very slow shifting toward smaller wavelength values (see Figure 4). This period of time almost agrees with the previously

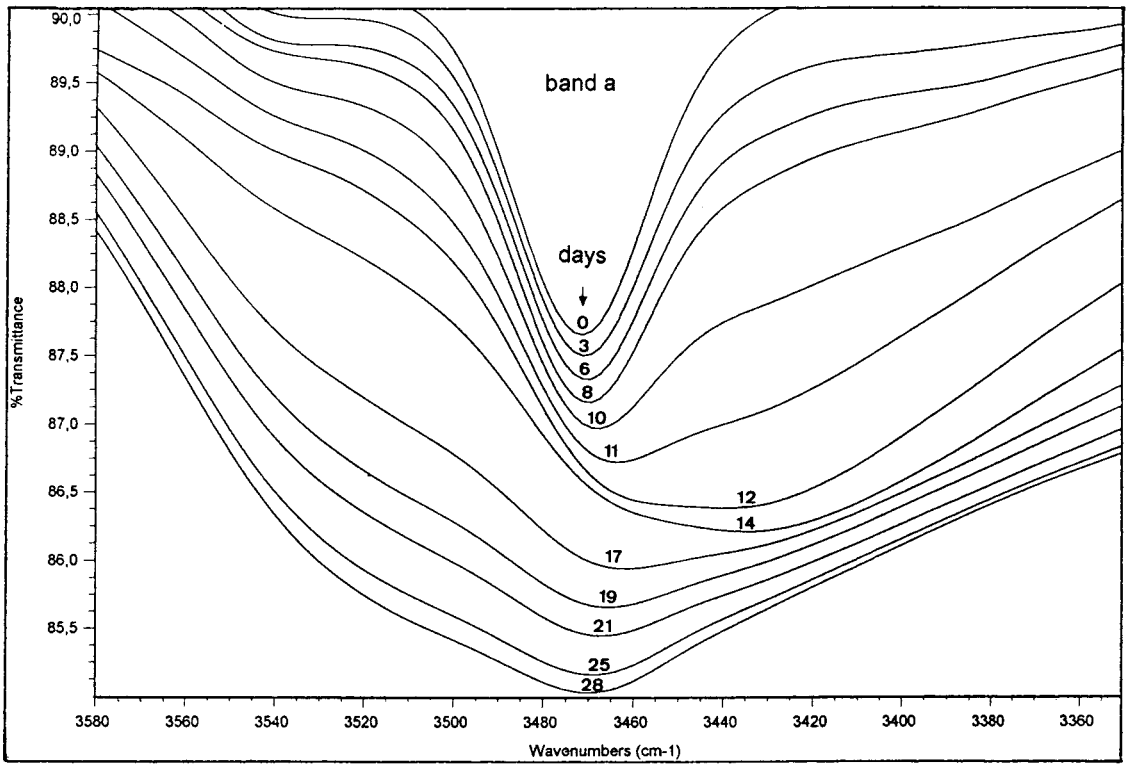

Figure 2. Region between 3580 and 3350 cm^{-1} of the infrared spectra of the sesame oil sample (F) at different days under oxidative conditions.

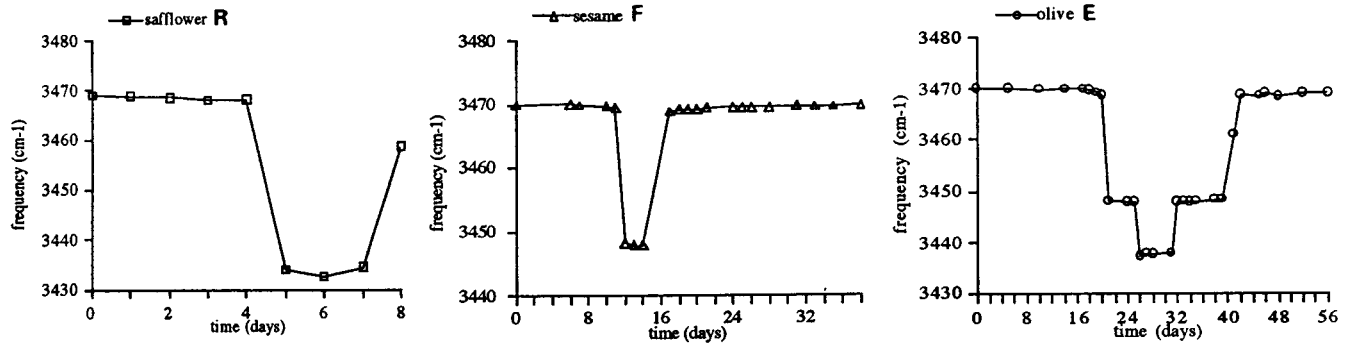

Figure 3. Frequency values of band **a** versus time for safflower (R), sesame (F), and olive (E) oil samples under oxidative conditions.

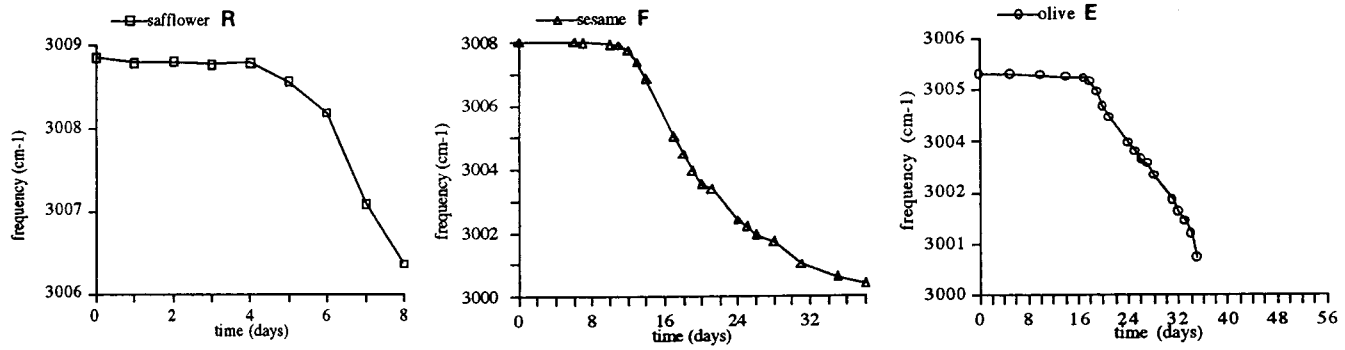

Figure 4. Frequency values of band **c** versus time for safflower (R), sesame (F), and olive (E) oil samples under oxidative conditions.

named FS or, in some oil samples rich in oleic acyl groups, can be considered a little shorter. After this period a clear and pronounced diminution in the frequency value of this band is produced; the beginning of this clear diminution in the value of the frequency of this band agrees with the appearance of the hydroperoxide functional group band. As the oxidation process advances, the frequency of the cis double bond stretching vibration band reaches smaller values at a rate that is basically dependent on the nature of the oil. This band in some oil samples, such as virgin olive, olive, peanut, and rapeseed oils, is transformed into a shoulder and in some cases disappears completely before the polymerization of the sample, showing the total extinction of the cis double bond in the sample; typical end values of the frequency of this band before its transformation

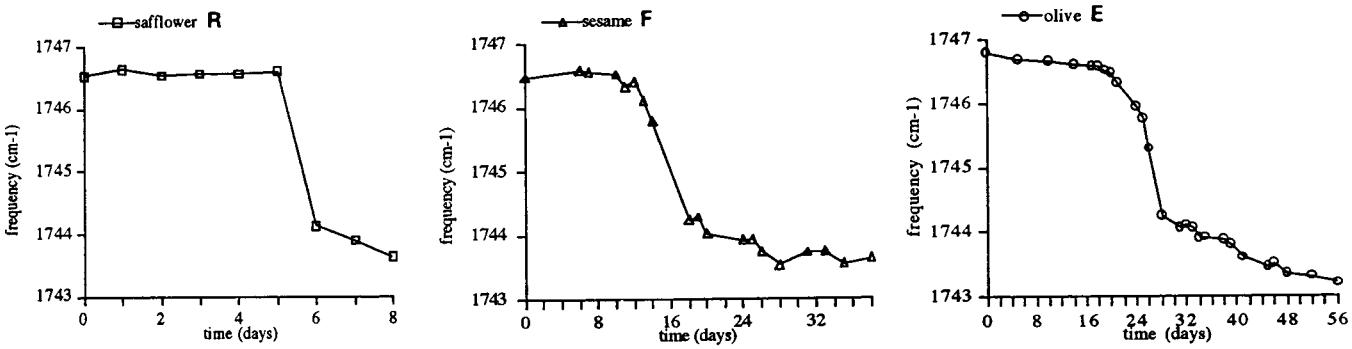

Figure 5. Frequency values of band **i** versus time for safflower (R), sesame (F), and olive (E) oil samples under oxidative conditions.

into a shoulder are near 3001 cm^{-1} in the most oxidation-resistant oil samples. However, in oil samples with a high proportion of linoleic and/or linolenic acyl groups, in which the oxidation is produced very quickly, the band never disappears, and although its frequency is shifted to lower values, it never reaches such low values as are obtained in the oxidation of oil samples rich in oleic acyl groups.

In conclusion, oil samples that show frequency values of the **c** band smaller than is typically due to its composition are in advanced stages of the oxidation process; the smaller the frequency value of the band is, the more advanced is the oxidation state of the sample.

The following bands in this region appear at approximately 2925 and 2854 cm^{-1} (bands **e** and **f**, respectively in Figure 1). They are due to the symmetric and asymmetric stretching vibration bands of the aliphatic CH_2 functional groups (Bellamy, 1975). The CH_3 functional groups give symmetric and asymmetric stretching vibration bands at approximately 2962 and 2872 cm^{-1}; the first causes a shoulder **d** in the spectrum, and the second is responsible for the diminution of the valley between the two bands due to CH_2 functional groups. When an oil sample is kept under oxidative conditions, the frequency of the band at 2854 cm^{-1} remains almost invariable during a period of time. This period of time is a little greater than FS in some oil samples and practically agrees with FS for other oil samples. After this period, the frequency of this band increases slowly from 2854 to 2855 cm^{-1} in most cases. These small changes in the frequency values of these functional groups are due to surrounding chemical changes as a consequence of the oxidation process.

Very weak bands, **g** and **h**, at approximately 2678 and 2730 cm^{-1} are present in all nonoxidized and oxidized oil samples. The assignment of these bands is difficult; aldehydes frequently show two moderate absorption bands in the 2830−2695 cm^{-1} region, and carboxylic acid dimers can display bands between 3300 and 2500 cm^{-1} (Silverstein et al., 1974; Günzler and Böck, 1975). During the oxidation process some changes are observed in the frequency of these bands, but they are not relevant.

Changes in the Region between 1800 and 1500 cm^{-1}. In oil samples a very intense absorption due to the ester carbonyl functional group of the triglycerides causes a band **i** at ∼1746 cm^{-1}. Under oxidative conditions, the frequency of this band remains practically unchanged for a period of time that is a little longer than the FS period in most of the oil samples studied. Afterward, the frequency of this band begins to decrease at different rates depending on the nature of the oil; the variation is pronounced from 1746 to 1743 cm^{-1} in most cases. This change can be associated with the appearance of saturated aldehyde functional groups (Frankel, 1980; Hamilton, 1989) or secondary oxidation products that cause an absorption at 1728 cm^{-1} which overlaps with the band of the ester functional group; the time interval from the beginning of the oxidation experiment to frequency values of this band under 1744 cm^{-1}, due to the presence of saturated aldehyde functional groups in appreciable proportions, is coincident in most cases with the total time from the beginning of the experiment to the end of the SS interval. In short, the generation of alcohol functional groups in appreciable proportions in these samples agrees with the generation of aldehyde functional groups, also in appreciable proportions. However, in olive and virgin olive oils, the frequency of this band reaches values below 1744 cm^{-1} before the end of the SS interval, which is very long due to the low oxidation rate. Figure 5 shows the evolution of the frequency of this band versus the time in safflower (R), sesame (F), and olive (E) oil samples. The rate of change in the frequency of this band in each oil sample could be considered as a measure of the generation rate of aldehydes, which are responsible for the oxidized oil odor. From this result it is evident that the generation of secondary oxidation products begins a little later than the generation of hydroperoxide functional groups, but its concentration is significant in most of the cases when the concentration of hydroperoxides begins to decrease, that is to say, at the end of the SS interval, except for olive and virgin olive oil samples. The presence of the band at 1728 cm^{-1} is not detectable by eye, but it is detectable from the subtraction between the spectrum of an oxidized oil sample and the spectrum of the same oil at the beginning of the oxidation experiment. On the other hand, it must be pointed out that some authors have indicated that absorptions due to hydrogen bonding of hydroperoxides and alcohols with the triglyceride ester carbonyl group contribute to the band near 1728 cm^{-1}, although the vibrational mode for this kind of absorption has not been explained (Dubois et al., 1996).

In conclusion, oil samples with frequency values for band **i** lower than 1746 cm^{-1} are involved in an oxidation process. The more this process is advanced, the smaller is the frequency value of this band; values under 1744 cm^{-1} are due to appreciable proportions of aldehyde functional groups in the sample. The more quickly the frequency of band **i** shifts to lower values, the faster is the oxidation process and the lower will be the oxidative stability of the sample.

In all nonoxidized oil samples a shoulder **j** near 1700 cm^{-1} is observed, which disappears as the oxidation process advances. This shoulder could be due to free

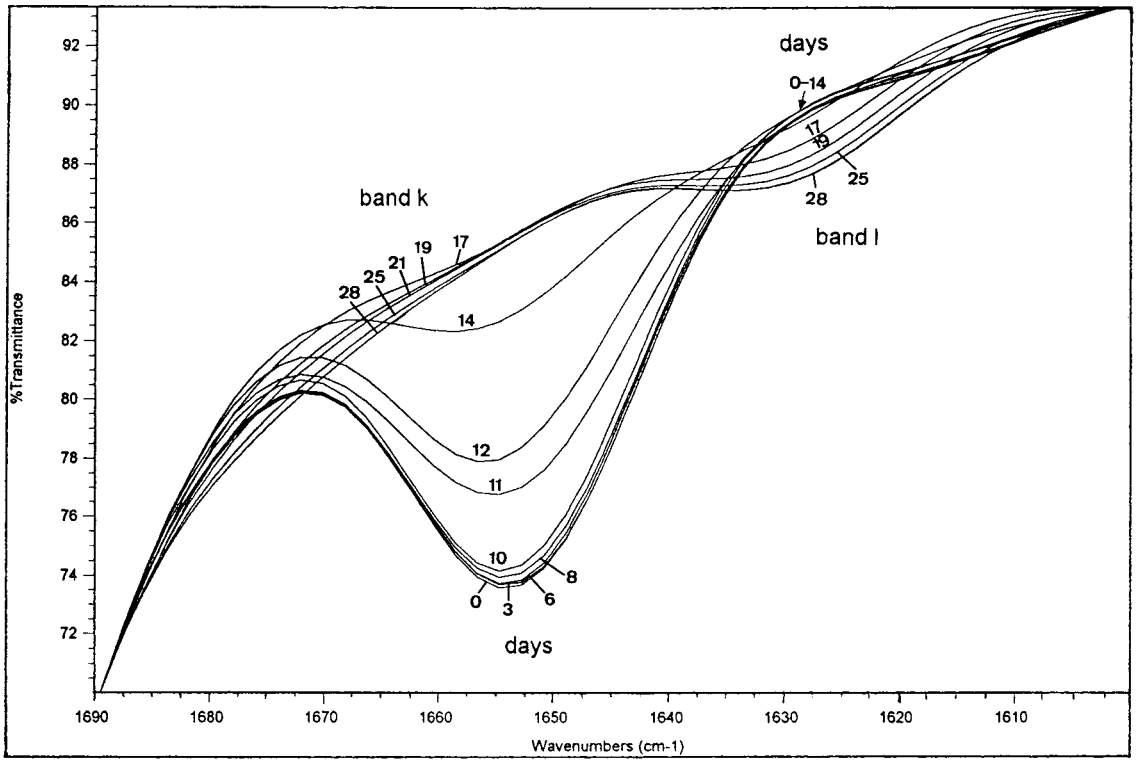

Figure 6. Region between 1690 and 1600 cm^{-1} of the infrared spectra of the sesame oil sample (F) at different days under oxidative conditions.

fatty acids present in all oil samples in small proportions and probably disappears as a consequence of overlapping with neighboring bands.

Changes during the oxidation process are also observed in the region between 1620 and 1670 cm^{-1}. Nonoxidized oils show a very weak band, **k**, at ~1654 cm^{-1}. This band has been associated with the stretching vibration of the carbon−carbon double bonds of *cis*-olefins. The intensity of this band diminishes as the oxidation process advances and in all cases disappears practically when the frequency of **i** band reaches values below 1744 cm^{-1}, that is, at the end of the SS interval for all samples except olive and virgin olive oils. Again, the faster the oxidative process, the faster the disappearance of this band.

On the other hand, as the oxidation process advances, a very weak band, **l**, appears at ~1630 cm^{-1}. It has been assigned to α,β-unsaturated aldehydes and ketones (Van de Voort et al., 1994b). The appearance of this band is produced at the end of the SS interval for all samples except for olive and virgin olive oils, in which it is produced during the SS interval in agreement with changes in band **i**. In Figure 6, the region between 1690 and 1600 cm^{-1} of the infrared spectra of sesame oil corresponding to different days of the oxidation process, showing the **k** and **l** bands, is given.

Changes in the Region between 1500 and 1000 cm^{-1}. In nonoxidized oil samples there appear bands **m** and **p** (see Figure 1), due to bending vibrations of CH_2 and CH_3 aliphatic groups near 1464 and 1377 cm^{-1}. The latter is specific to CH_2 groups, and the band at 1464 cm^{-1} is due to asymmetric bending vibrations of CH_2 and CH_3 groups. Changes observed in the frequency of these bands during the oxidation process are not significant.

The following band in the infrared spectrum is band **n** near 1417 cm^{-1}, present in all oil samples, tentatively assigned to rocking vibrations of CH bonds of cis-disubstituted olefins. The frequency of this band remains unchanged throughout the FS period and afterward diminishes to values near 1416 cm^{-1} in most cases. Despite this change in the frequency value not being large, it is very sharp.

Another very weak band of the spectrum of oil samples in this region is band **o** near 1399 cm^{-1}. The frequency of this band, difficult to assign, has been proved to be closely related, in a direct way, to the proportion of the oleic acyl groups in the sample (Guillén and Cabo, 1997a), and some authors have associated it with bending in plane vibrations of CH cis olefinic groups (Dahlberg et al., 1997). The evolution of the frequency of this band during the oxidation process depends greatly on the nature of the sample (see Figure 7); in the IR spectra of safflower, walnut, soybean, and corn oil samples, the frequency of this band remains practically unchanged, near 1397 cm^{-1}, during the FS period, afterward reaching lower values near 1396 cm^{-1}, and finally disappearing. However, in two different sunflower oil samples and in an oil sample of unspecified vegetable origin, the frequency of this band remains unaltered, near 1397 cm^{-1}, during the FS period and afterward reaches a minimum value of near 1396 cm^{-1}, later increasing to values near 1400 cm^{-1}, higher than the original value. During the FS period oil samples with a significant proportion of oleic acyl groups, such as sesame, rapeseed, and peanut oil samples, maintain the frequency of this band practically constant, near 1398, 1400, and 1401 cm^{-1}, respectively, and afterward the value of this frequency increases to reach values near 1400 and 1403 cm^{-1}; finally, in olive and virgin olive oils the frequency of this band remains unchanged at values near 1402 cm^{-1} during the FS period, and afterward this frequency decreases to reach values near 1399 cm^{-1}. From these results it can be concluded that,

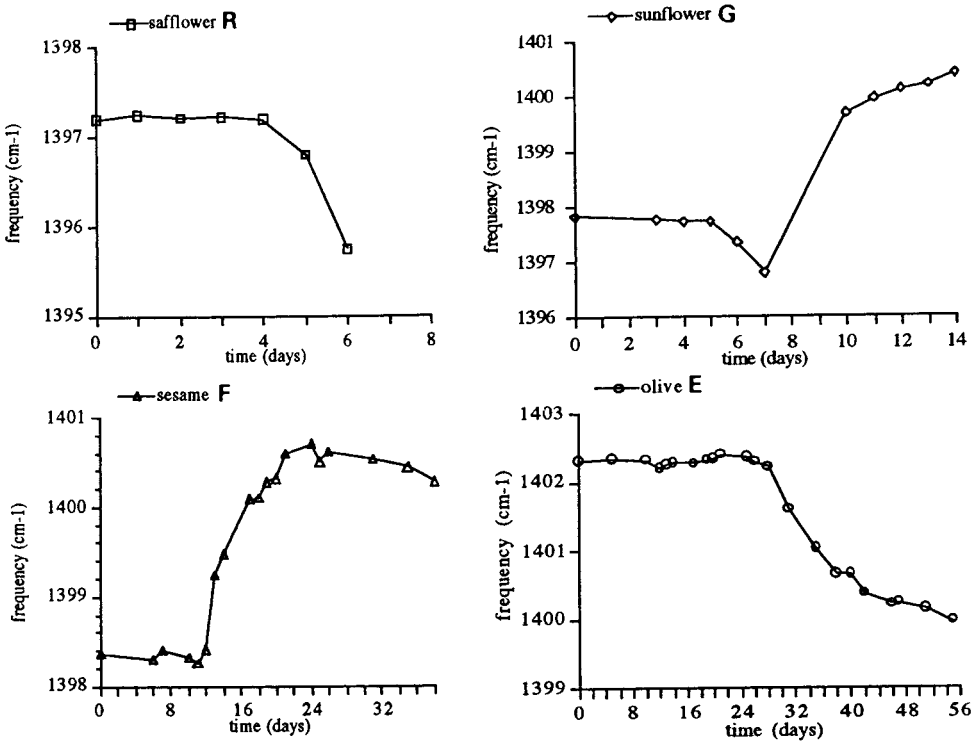

Figure 7. Frequency values of band **o** versus time for safflower (R), sunflower (G), sesame (F), and olive (E) oil samples under oxidative conditions.

although the frequency of this band remains unchanged in all oil samples during the FS period, its variations from FS period to the end of the process are very different in the several samples and allow their classification into four groups. Figure 7 gives variations of the frequency of band **o** with time in oils of the four mentioned groups.

Two other bands present in all oil samples are bands **r** and **s** near 1238 and 1163 cm^{-1}, respectively. The frequencies of both bands have been proved to be related to the proportion in the sample of saturated acyl groups (Guillén and Cabo, 1997a). The frequencies of both bands suffer similar changes during the oxidation process, although the changes are sharper in the band near 1163 cm^{-1}. In all oil samples studied, the frequency of this band remains unchanged, near 1163 cm^{-1}, throughout the FS period and afterward increases to near 1166 or 1167 cm^{-1} with a rate that is dependent on the nature of the oil. In oil samples with low oxidative stability, the frequency of this band reaches the latter value very quickly, and in oil samples with higher oxidative stability, the frequency of this band reaches the cited value more slowly.

As in other bands mentioned, band **t** near 1119 or 1120 cm^{-1} shows an almost constant frequency value during the FS period, in all oil samples, and afterward, this value diminishes sharply to lower values. In nonoxidized oils the frequency value of this band has been found to be related, in an inverse way, to the proportion of saturated acyl groups in the sample (Guillén and Cabo, 1997a), and it is logical to observe a decrease in the value of the frequency of this band as a consequence of the oxidation process due to the extinction of unsaturation in the oil samples. The rate at which the value of the frequency of bands **s** and **t** begins to change is another measure of oxidative stability.

The frequency of band **u** near 1099 cm^{-1} does not generally suffer large variations except in oil samples rich in oleic acyl groups. As the oxidation process advances, the frequency of this band diminishes and reaches a minimum value; the time interval in which this minimum value is reached agrees with the beginning of the SS period in all samples, and afterward the frequency of this band acquires higher values. This increase is more noticeable in oil samples rich in oleic acyl groups, such as rapeseed, peanut, olive, or virgin olive oil samples in which the initial values of the frequency of this band are near 1098 or 1096 cm^{-1} and the final value is near 1099 cm^{-1}. The minimum observed in most of the samples is not present in olive and virgin olive oils; in these latter samples the frequency of band **u** remains unchanged until the beginning of the SS interval and then makes a continuous increase to the end values.

Band **v** near 1033 cm^{-1} is present in all oil samples; the frequency of this band changes with the advance of the oxidation process, but no patterns have been observed in these changes.

Changes in the Region between 1000 and 500 cm^{-1}. In the infrared spectrum of some nonoxidized oil samples such as walnut, soybean, safflower, corn, and sesame oil samples, there is a weak band **w** near 983 or 985 cm^{-1} depending on the nature of the oil. In these samples, as Figure 8 shows, the frequency of this band increases as the oxidation process advances to reach a maximum value near 988 cm^{-1}, which coincides with the beginning of the SS period, or when the value of the frequency of band **a** is near 3448 cm^{-1} due to an appreciable concentration of hydroperoxide groups. In addition, the maximum absorption of this band is produced when the maximum frequency values are near 988 cm^{-1} and just when the hydroperoxide functional group band appears.

In other nonoxidized oil samples such as rapeseed, peanut, sunflower, olive, and virgin olive oil as well as two different oil samples of unknown vegetable origin,

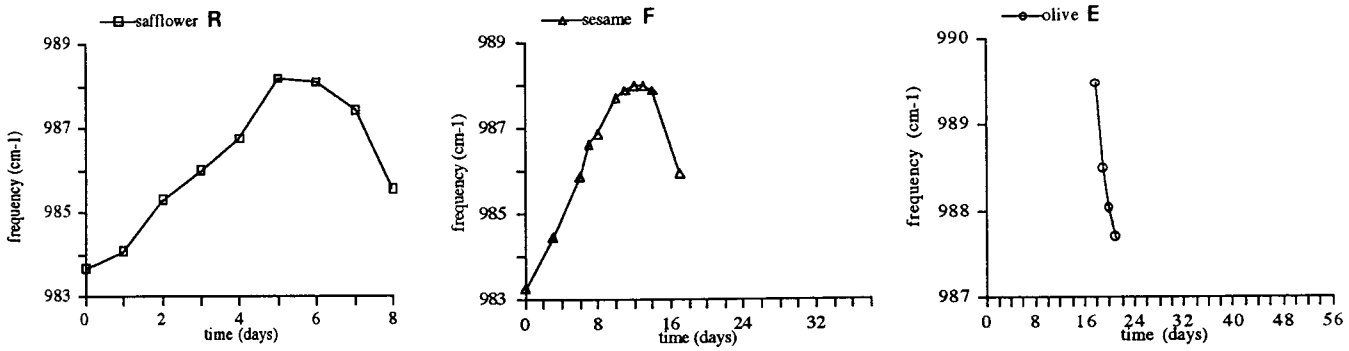

Figure 8. Frequency values of band **w** versus time for safflower (R), sesame (F), and olive (E) oil samples under oxidative conditions.

this band **w** is absent from the spectrum, but as the oxidation process advances this band appears. The appearance of this band is produced before the end of the FS period and remains to approximately the end of the SS period except in olive and virgin olive oil samples, in which this band exists discretely only in the first stages of hydroperoxide formation (see Figure 8).

This single band **w** near 988 cm^{-1} has been associated with bending vibrations of CH trans,trans-conjugated olefinic double bonds (Chan and Levett, 1977b). It has been assumed that isomerizations are produced during the oxidation process (Hamilton, 1989), and from the results here obtained it is evident that the trans,trans-conjugated dienes are formed at the end of the FS period and that they disappear when the concentration of hydroperoxide functional groups begins to decrease, in most of the samples studied at the end of the SS period, showing that compounds with this isomerization are intermediate compounds in the oxidation process. Olive and virgin olive oil samples are an exception; in these samples the band near 989 cm^{-1} appears only at the end of the FS period and at the beginning of the SS period, and its very rapid extinction shows that the trans,trans-conjugated double bonds formed play an important role only in the beginning of the hydroperoxide functional groups generation. Probably due to the small concentration of linoleic and linolenic acyl groups in these latter oils, the formation of trans,trans-conjugated double bonds and their participation in the oxidation process are relegated to this small interval of time (see Figure 8).

Also, a band near 988 cm^{-1} together with another band near 953 cm^{-1} has been assigned to cis,trans-conjugated olefinic double bonds (Chan and Levett, 1977b); although these two bands have been found in the oxidation of the lipids of other food samples by Guillén and Cabo (Study of the Effects of Smoke Flavorings on the Oxidative Stability of Lard by FTIR Spectroscopy, unpublished results), in the oxidation of the edible oils here studied the band near 953 cm^{-1} was not detected, showing that isomer cis,trans-double bonds were absent from this process.

With the exception of virgin olive oil, which shows only a shoulder near 967 cm^{-1}, the other oil samples studied have a small band **x** at this wavenumber due to the bending vibrations of CH functional groups of isolated *trans*-olefins present in their composition. As the oxidation process advances and the absorption of the band at 988 cm^{-1} decreases, the absorption and the frequency of the band at 967 cm^{-1} increase, at different rates for different periods of time, depending on the nature of the oil, to reach end values near 973 cm^{-1} in

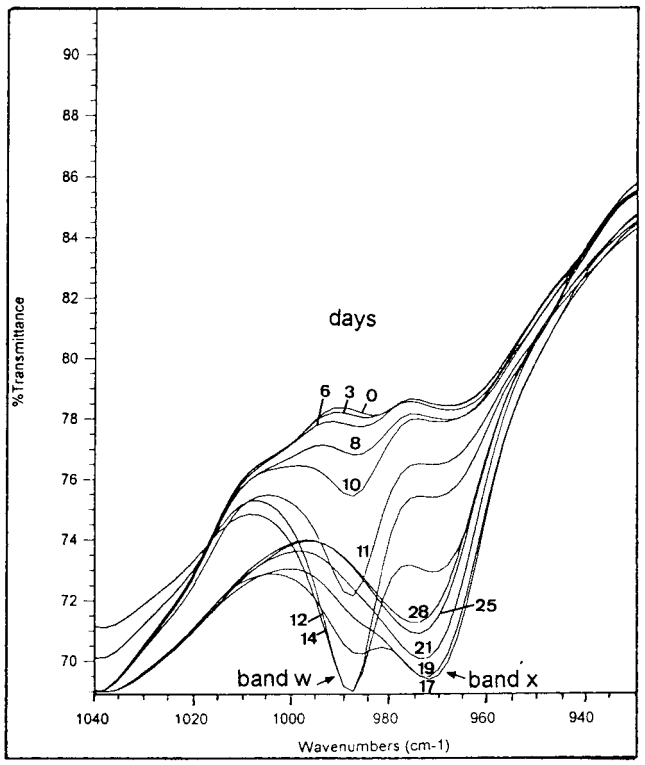

Figure 9. Region between 1040 and 930 cm^{-1} of the infrared spectra of the sesame oil sample (F) at different days under oxidative conditions.

oil samples rich in linoleic or linolenic acyl groups or near 976 cm^{-1} in oil samples rich in oleic acyl groups. The initial band near 967 cm^{-1} is associated with acyl groups having isolated *trans*-olefins; bands at higher wavenumbers 973 and 976 cm^{-1} could be assigned to secondary oxidation products such as aldehydes or ketones supporting isolated trans-double bonds. In samples with a significant proportion of linolenic acyl groups such as walnut, rapeseed, and soybean oils, the frequency of this band begins to increase from the beginning of the experiment; however, in the other samples there is a period of time less than FS in which the frequency of this band does not change, showing that the first change in the oxidation experiment observable by FTIR is due to an isomerization process and is observed in this band. Figure 9 shows the observed changes in bands **w** and **x** of the infrared spectra of a sesame oil sample as the oxidation process advances. In conclusion, an oil sample having an infrared spectrum showing band **w** near 988 cm^{-1} is in an oxidation process, at the end of the FS period, or at the beginning

of the SS period. Oil samples with infrared spectra showing band **x** with frequency values higher than 967 cm^{-1} are in an oxidation process, which is more advanced when the frequency of the band is higher.

A band absent only in virgin olive and olive oil samples is band **y** near 914 cm^{-1} (see Figure 1) and is difficult to assign. The changes observed in the frequency of this band as the oxidation progresses are not relevant; however, this band practically disappears in all samples studied when the maximum frequency of band **a** returns to near 3468 cm^{-1}, that is, when the concentration of hydroperoxide functional groups begins to decrease or when the production of secondary oxidation products such as alcohols and aldehydes begins to be important. For this reason, its extinction rate, in those samples in which it is present, could be considered as another useful parameter for measuring the rate of appearance of secondary oxidation products in significant concentrations.

Very weak bands are present in most of the oil samples studied, near 873 and 846 cm^{-1}, and are difficult to assign. As the oxidation advances these bands disappear, and at the beginning of the last oxidation stage, that is, at the end of the SS period, there appears a very weak band near 886 cm^{-1}, which could be associated with secondary oxidation products; in virgin and olive oils this latter band appears at the beginning of the SS period. In these oils appears a band near 893 cm^{-1}, instead of the band at 886 cm^{-1}.

Finally, all oil samples have a band near 723 cm^{-1}. This band has been associated with the overlapping of the methylene rocking vibration and the out-of-plane bending vibration of cis-disubstituted olefins (Silverstein et al., 1974; Günzler and Böck, 1975). During the FS period the frequency value of this band remains unchanged; however, it increases as oxidation products are formed, with end values $\sim$1 cm^{-1} above the initial value in all samples studied.

From the results here obtained it can be concluded that FTIR spectroscopy is an adequate technique for following the changes produced in an edible oil sample during an oxidation process. The changes observed in the frequency values of most of the bands of the spectra throughout the oxidation experiment provide accurate information about the different stages of the process. It must be taken into account that the spectra were recorded from the pure sample deposited between two KBr disks, the frequency values are given automatically by the corresponding command of the equipment, and the preparation of the sample, registration of the spectrum, and obtention of the frequency data took 2−3 min. It has been observed that the oxidation process of the 13 samples follows the same patterns, and the frequency values of most of the bands provide information of the oxidation degree of each oil sample and for this reason also allow one to evaluate the oxidative stability of different oil samples in a simple, fast, and accurate way.

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