Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 573–574 (2006) 459–465
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

Applications of Fourier transform-infrared spectroscopy to edible oils

N. Vlachos, Y. Skopelitis, M. Psaroudaki, V. Konstantinidou, A. Chatzilazarou, E. Tegou *

Food Technology Department, Faculty of Food Technology and Nutrition, Technological Educational Institute of Athens, Ag. Spyridonos Str., 12210 Egaleo, Athens, Greece

Received 27 November 2005; received in revised form 8 May 2006; accepted 10 May 2006
Available online 17 May 2006

Abstract

Recent developments in Fourier transform infrared (FT-IR) spectroscopy instrumentation extend the application of this technique to the field of food research, facilitating particularly the studies on edible oils and fats. In this work, FT-IR spectroscopy is used as an effective analytical tool in order: (a) to determine extra virgin olive oil adulteration with lower priced vegetable oils (sunflower oil, soyabean oil, sesame oil, corn oil) and (b) to monitor the oxidation process of corn oil samples undergone during heating or/and exposure to ultraviolet radiation. A band shift observed at 3009 cm^{-1} assigned to the C—H stretching vibration of the *cis*-double bond, allows the determination of extra virgin olive oil adulteration. Changes in the 3050–2800 and 1745 cm^{-1} spectral region appear after heating at elevated temperatures and aid the oxidation process monitoring. In addition, an analytical technique for the measurement of carbonylic compounds in oils, produced after heating, is applied. The possible antioxidant effect of oregano is also discussed.

Keywords: Fourier transform infrared (FT-IR) spectroscopy; Edible oils; Olive oil; Adulteration; Oxidation

1. Introduction

The production of olive oil constitutes a significant proportion to the income of farmers of Southern Europe and contributes considerably to hindering depopulation of countryside. In this way, olive oil production is a promising factor to the viability of agricultural economy. Nevertheless, the expansion of olive oil adulteration with various lower price of money vegetable oils in global market, comprises a great hazard not only for the economic development and prosperity of those communities but also for the health and safety of olive oil consumers.

In addition, the oxidative stability of oils, i.e. their resistance to the oxidation process, is an important indicator of performance and shelf-life, that depends on the composition of the sample and the conditions the sample is subjected to. Normally, an oil sample is oxidized when subjected to air or oxygen flow, heating, exposure to light, catalysers, etc. The oxidative conditions influence the mechanism of the oil degradation process. Although other degradation mechanisms are also possible, the oil degradation process has generally been established as being a free radical mechanism yielding hydroperoxides, also called primary oxidation products. Consequently, these in their turn degrade to secondary oxidation products: aldehydes, ketones, lactones, alcohols, acids, etc. The methods used to determine the degree of the oxidation process are mostly related to the measurement of the concentration of primary or secondary oxidation products or both, or to the amount of oxygen consumed during the process. Among those, peroxide value (P.V.), which measures hydroperoxide concentration, is one of the most popular.

Over the past 15 years, as a result of the development of Fourier transform infrared (FT-IR) spectroscopic instrumentation, the application of this technique expanded in food research and particularly has become a powerful analytical tool in the study of edible oils and fats. FT-IR spectroscopy is a rapid, non-destructive technique with minimum sample preparation necessary. It allows the qualitative determination of organic compounds as 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. Moreover, FT-IR spectroscopy is an excellent tool for quantitative analysis as the intensities of the bands in the spectrum are proportional to concentration

* Corresponding author. Tel.: +30 2104614485/6937949497; fax: +30 2104612702.
E-mail address: etegou@yahoo.com (E. Tegou).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2006.05.034

(i.e. Beer's law is obeyed). Mid-infrared spectra have been used to characterize edible oils and fats, because they differentiate 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 [1–7].

Over the past few years, FT-IR spectroscopy combined with discriminant analysis and partial least-squares analysis, has been successfully used in order to quantify adulteration of extra virgin olive oil with refined oils and different types of vegetable and nut oils [8–11]. As reported by Ozen et al., adulteration of virgin olive oil with hazelnut oil could be detected only at levels of 25% while the detection limit for the adulteration with dietary supplement oils was lower (2%) [12,13].

Moreover, the changes observed in the frequency data of some bands and also in the ratios of absorbances of the FT-IR spectra during the oxidation process have been studied recently [14,15]. The use of ratios of absorbance values instead of absolute absorbance values is preferable because the former eliminate all variable circumstances involved in the sample preparation, acquisition of the spectrum and thickness of the sample.

In the present study, FT-IR spectroscopy is used for the determination of extra virgin olive oil adulteration with various vegetable oils (sunflower oil, soyabean oil, sesame oil, corn oil) and also for the monitoring of the oxidation process of corn oil. First, an evaluation of a typical FT-IR spectrum of a corn oil sample is presented. A band shift observed at 3009 cm^{-1} assigned to the C–H stretching vibration of the *cis*-double bond, allows the determination of extra virgin olive oil adulteration. For the monitoring of the oxidation process, corn oil samples are heated or/and exposed to ultraviolet (UV) radiation and FT-IR spectra are collected at various temperatures. The noticed differences in the specific spectral bands (3050–2800 and 1745 cm^{-1}) are plotted and discussed. In addition, an analytical technique for the measurement of carbonylic compounds produced due to oxidation is applied [16]. The results are compared to those obtained by the peroxide value method [17].

2. Experimental

2.1. Samples collection

For the study of extra virgin olive oil adulteration with vegetable oils, edible oils purchased from local supermarkets were used. More specifically, two types of olive oils were used: extra virgin olive oil and olive-kernel oil. Four types of vegetable oils were used: sunflower oil, soyabean oil, sesame oil and corn oil.

For the study of oxidation process, corn oil and extra virgin olive oil was used. The possible antioxidant effect of oregano in oil was tested. Oregano was bought from local supermarkets.

2.2. Adulteration experiments

For the adulteration studies, blends of extra virgin olive oil and various kinds of vegetable oils were prepared by mixing these oils in a total volume of 10 mL. Extra virgin olive oil was adulterated with vegetable oils at 2–90%, v/v.

2.3. Sample oxidation

Fifty millilitre of corn oil were placed in metallic beakers and heated on an electric device, stirring manually with a glass rod. A thermometer was introduced into the beaker to monitor the sample temperature. To mimic the oil oxidation process during frying, the samples were heated up to various temperatures from 130 up to 275 °C. The heating duration was held constant at 30 min. In the experiments that oregano was added to oil, the concentration used was 0.05% w/w.

2.4. Exposure to ultraviolet radiation

The samples were exposed to the radiation of an Oriel deep ultraviolet illuminator. This illuminator is a mercury–xenon lamp with an emission spectrum between 220 and 440 nm. Five millilitre of corn oil were placed under the lamp in uncovered polystyrene Petri dishes and exposed for 1, 2, and 3 h corresponding to 1.6, 2 × 1.6 and 3 × 1.6 J cm^{-2}, respectively.

2.5. FT-IR spectra acquisition

A Perkin-Elmer Spectrum RXI FT-IR spectrophotometer equipped with a deuterated triglycerine sulphate (DTGS) detector was used to collect FT-IR spectra with a resolution of 4 cm^{-1} at 64 scans. The data interval provided by the instrument for a resolution of 4 cm^{-1} is 1 cm^{-1}. A small quantity (∼2 μL) of the sample was deposited with the use of a Pasteur pipette between two well-polished KBr disks, creating a thin film. Duplicate spectra were collected for the same sample. All spectra were recorded from 4000 to 400 cm^{-1} and processed with the computer software program Spectrum for Windows (Perkin-Elmer).

2.6. FT-IR quantitative analysis

For the construction of the calibration curve, corn oil and internal standard *n*-butanal (analytical grade) were weighed on an analytical scale and mixed in a test tube. The mixture was shaken manually to ensure total homogenization. The proportion in weight of *n*-butanal in the prepared samples ranged from 2 to 40 %, w/w (C_b). The spectra were integrated in the regions between 1726 and 1700 cm^{-1} (A_1) and 1840–1743 cm^{-1} ($A_{1/2}$) employing a baseline between 1840 and 1674 cm^{-1}. Finally, the quotient $A_1/A_{1/2}$ was calculated, where each quotient corresponds to a specific mass ratio C_b/C_a (C_a: total mass).

3. Results and discussion

3.1. Evaluation of an FT-IR spectrum

First, as a descriptive example a corn oil FT-IR spectrum is presented in Fig. 1. In Table 1 the analytical evaluation of this spectrum is given [1].

3.2. Determination of adulteration

FT-IR spectra of various oil samples show that there exist notable differences in the band around 3006 cm^{-1} assigned to

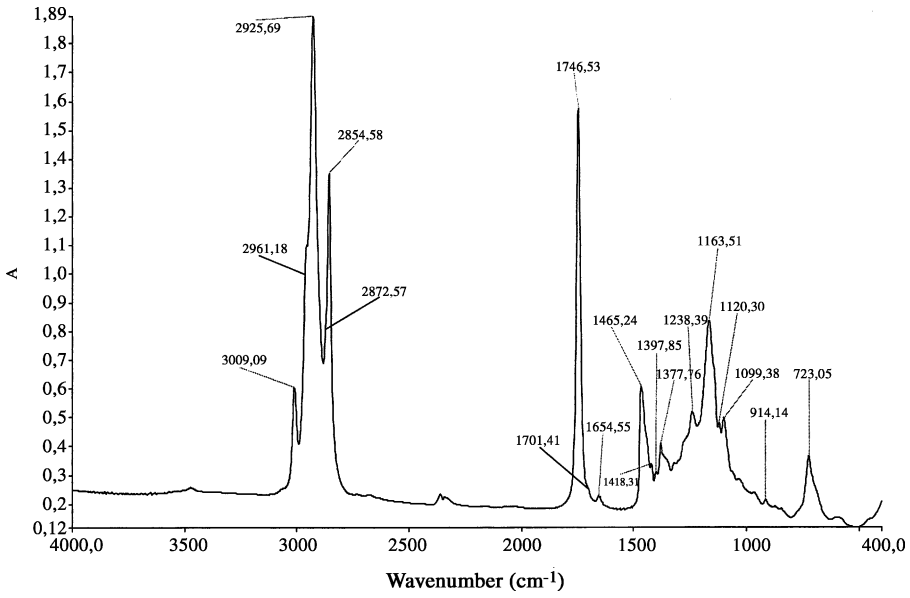

Fig. 1. FT-IR spectrum of a corn oil sample at room temperature (25 °C).

the C–H stretching vibration of the *cis*-double bond (=CH). The oil composition affects the exact position of the band and yields shifts when the proportion of the fatty acid changes. In this way, the value of this frequency in non-oxidized oil samples varies significantly from 3009 to 3006 cm^{-1}. For example, as depicted in Fig. 2, sunflower oil, soyabean oil, corn oil and sesame seed oil show a maximum absorbance at ~3009 cm^{-1} comparing to extra virgin olive oil that has a maximum of absorbance at ~3006 cm^{-1}. This is due to their composition, as vegetable oils contain higher proportion of linolenic or linoleic acyl groups whereas extra virgin olive oil consists higher proportion of oleic acyl groups (see Table 2 [18]). This is in accordance with similar observations made by Guillen and Cabo [1].

Moreover, in the inset of Fig. 2, as the sunflower oil is gradually mixed in various proportions with extra virgin olive oil, the clear shift of the 3009 cm^{-1} band, attributed to the C–H stretching vibration of *cis*-double bond, to the 3005 cm^{-1} band is noticed. Based on this observation, we set out an effort to quantify the percentage of the added vegetable oil to extra virgin olive oil. Fig. 3 shows that there exists a linear relation between the observed band shift and the percentage of the added vegetable oil.

In order to quantify further the adulteration, the maximum heights of the two bands at ~3006 and 2925 cm^{-1} were also used. The 2925 cm^{-1} band is attributed to the symmetric stretching vibration of the aliphatic CH_2 group. The calculated quotient of the two peak heights denotes the percentage of the hydrogen–carbon bond coupled by *cis*-double bond (=CH) present in the oil. The height of the 3006 cm^{-1} band for the extra virgin olive oil sample is obviously smaller than it is for all the types of vegetable oils, and changes according to the extent

Table 1
Evaluation of the FT-IR spectrum

1. Region of functional groups	
1a. Region of hydrogen's stretching	
3009 cm^{-1}	C–H stretching vibration of the *cis*-double bond (=CH)
2925 cm^{-1}, 2854 cm^{-1}	Symmetric and asymmetric stretching vibration of the aliphatic CH_2 group
2962 cm^{-1}, 2872 cm^{-1}	Symmetric and asymmetric stretching vibration shoulder of the aliphatic CH_3 group
1b. Region of double bond's stretching	
1746 cm^{-1}	Ester carbonyl functional group of the triglycerides
1700 cm^{-1}	Free fatty acids shoulder
1654 cm^{-1}	C=C stretching vibration of *cis*-olefins
1c. Region of other bonds deformations and bendings	
1465 cm^{-1}	Bending vibrations of the CH_2 and CH_3 aliphatic groups
1418 cm^{-1}	Rocking vibrations of CH bonds of *cis*-disubstituted olefins
1397 cm^{-1}	Bending in plane vibrations of CH *cis*-olefinic groups
1377 cm^{-1}	Bending vibrations of CH_2 groups
2. Fingerprint region	
1238 cm^{-1}, 1163 cm^{-1}	Stretching vibration of the C–O ester groups
723 cm^{-1}	Overlapping of the CH_2 rocking vibration and the out-of-plane vibration of *cis*-disubstituted olefins

Table 2
Composition of fatty acids in oils

Oils	Oleic acid, C18:1 (%)	Linoleic acid, C18:2 (%)	Linolenic acid, C18:3 (%)
Olive oil	64–83	3.5–16	0.2–1.8
Olive-kernel oil	64–83	3.5–16	0.2–1.8
Sunflower oil	14–35	55–75	<0.3
Soyabean oil	18–26	50–57	5.5–10
Corn oil	24–42	34–62	<2.0
Sesame seed oil	35–50	15–50	<1.0

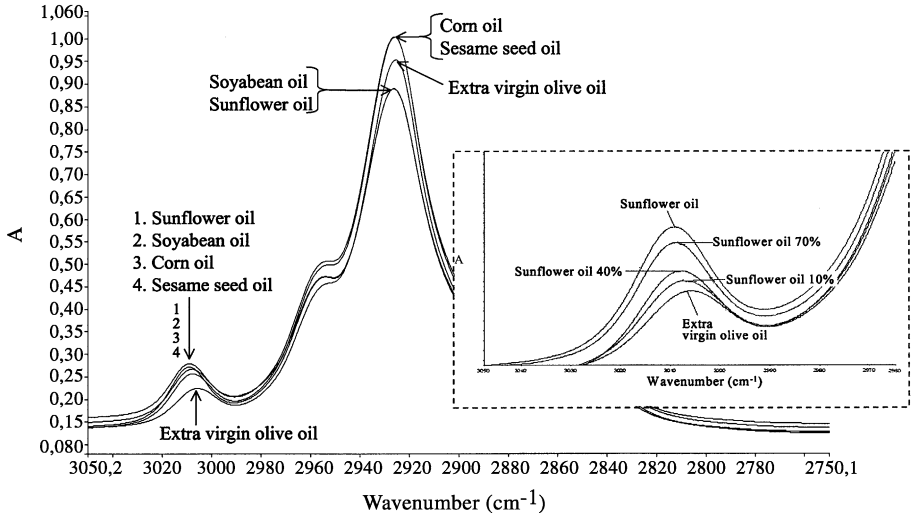

Fig. 2. Spectra of extra virgin olive oil and vegetable oils at 3050–2750 cm^{-1}. In the inset spectra of extra virgin olive oil adulterated with sunflower oil at ~3006 cm^{-1}.

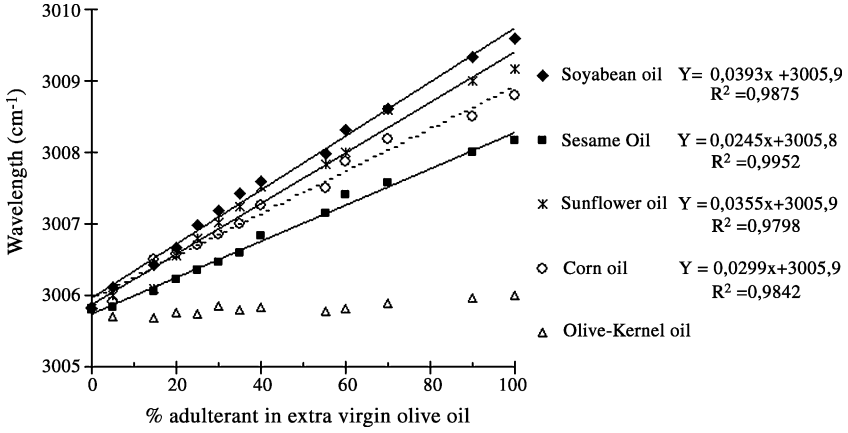

Fig. 3. The 3009 cm^{-1} band shift vs. the percentage of adulterant (soyabean oil, sesame seed oil, sunflower oil, corn oil, olive-kernel oil) in extra virgin olive oil.

of adulteration. When the percentage of added vegetable oil increases, the height of this band also increases, approaching the height that matches to the vegetable oil, when the adulteration is quite high. The 2925 cm^{-1} band height exhibits small changes and not in a specific way, as the 3006 cm^{-1} band. In Fig. 4, it is interesting to notice that the relation between the ratio of the peak heights and the adulteration percentage is linear with a correlation coefficient above 0.991. The lowest correlation coefficient (0.991) is observed for adulterations with sesame seed oil and corn oil while the highest correlation coefficient (0.996) is observed for sunflower oil and soyabean oil. In addition, the detection limit for oil adulteration is 9% if the adulterant is corn oil or sesame seed oil while it is lower (6%) if the adulterant is sunflower oil or soyabean oil. Detection limits are

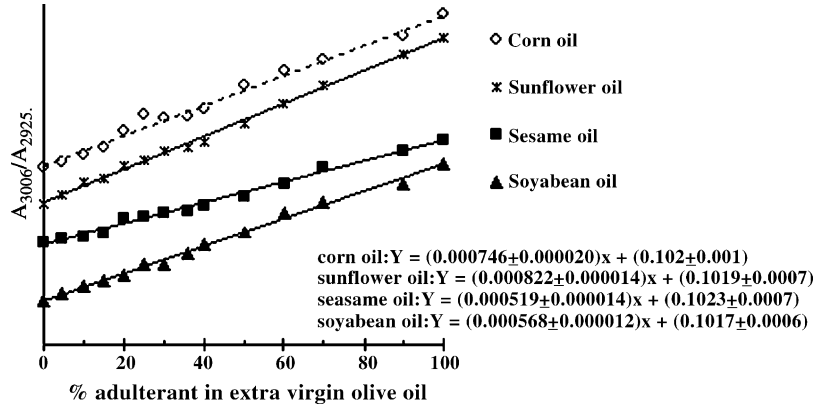

Fig. 4. Monitoring the adulteration of extra virgin olive oil with various vegetable oils with the use of the ratio A_{3006}/A_{2925}. Curves are arbitrarily offset.

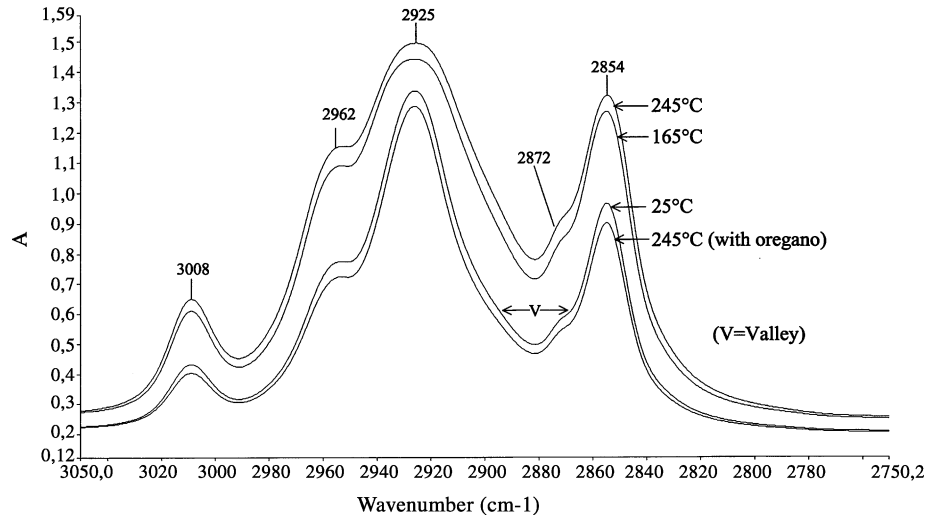

Fig. 5. Spectra of corn oil samples heated at 165 and 245 °C (with and without the addition of oregano) in comparison with the corn oil spectrum at room temperature.

calculated from the data of Fig. 4 from the following equation: LOD = (y_{LOD} − intercept)/slope, where: y_{LOD} = intercept + 3 Sb, LOD = Limit Of Detection, Sb = standard error of the regression statistics. The difference in the detection limits can be easily explained if we notice the difference in the composition of oils in oleic acid in Table 2. Sunflower oil and soyabean oil appear to have the lowest percentage of oleic acid, followed by corn oil, sesame seed oil and finally extra virgin olive oil. Moreover, for the sesame seed oil and corn oil, an interesting behavior is observed. The slope of the curve is lower for adulterant percentages lower than 15% and this is the reason for the lower correlation coefficient observed for these oils.

Finally, it was attempted to determine the adulteration of extra virgin olive oil with olive kernel oil. As can be seen in Fig. 3, no shift was observed in the 3006 cm^{-1} band for olive kernel oil. Unfortunately, the method developed in this work is not applicable to olive-kernel oil due to the similar acyl group composition of the oils (see Table 2).

3.3. Oxidation

3.3.1. Effect of heating

The spectral region between 3050 and 2740 cm^{-1} undergoes several changes during the oxidation process. As explained in Section 3.2, the frequency of the 3009 cm^{-1} band depends on the oil composition. It has been shown in Fig. 2 that oils with high proportion of linolenic or linoleic acyl groups show higher frequency data at this band than oils with high proportion of oleic acyl groups. In the oxidation experiments, corn oil was heated for 30 min at various temperatures and the changes in the FT-IR spectra were observed. The band at 2854 cm^{-1} and the shoulder at 2962 cm^{-1} increase their intensity but the band at 2925 cm^{-1} reduces its absorbance and increases its width as the temperature

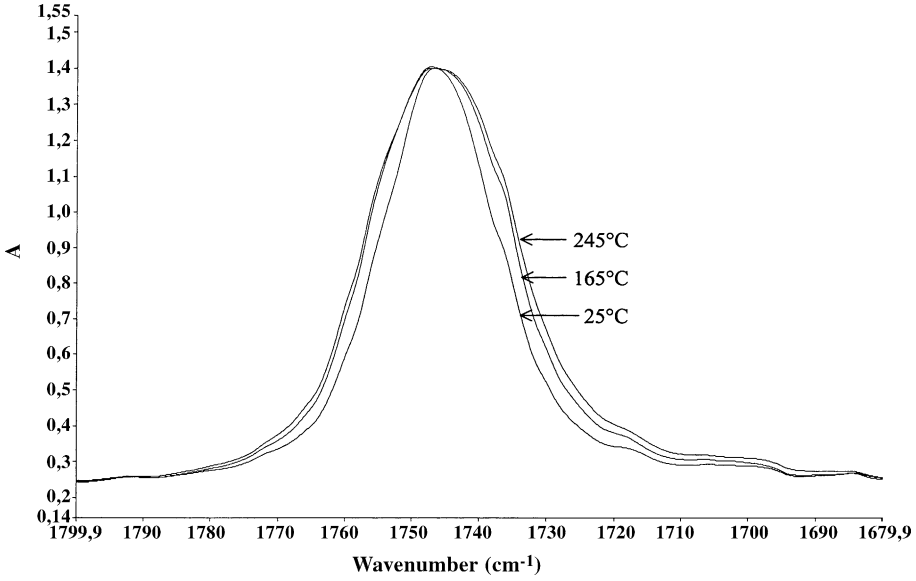

Fig. 6. Spectra of corn oil samples heated at 165 and 245 °C in comparison with the corn oil spectrum at room temperature.

gets higher. A shoulder at 2872 cm^{-1} is also formed attributed to the production of CH_3 groups (Fig. 5). A baseline shift is also observed.

At the same time, based on the observations of Fig. 5, a simple experiment was conducted in order to test the possible antioxidant effect of oregano in corn oil. Oregano and other essential oils such as basil, cinnamon, clove, nutmeg and thyme, traditionally used for their aromatic properties in the preparation of Mediterranean food, have been shown to exhibit good properties as antioxidants in a recent work by Tomaino et al. [19]. Other extracts proven to delay the oxidation process of oils during frying are from rosemary, sage and tea leaves [20–22].

Two corn oil samples with and without oregano added were heated at the same high temperature (245 °C) for 30 min, and the FT-IR spectra were recorded. Fig. 5 shows a clear reduction of the "valley" between the 2925 and 2854 cm^{-1} bands for the sample that was heated without containing oregano. On the contrary, for the oil sample that contained oregano during heating, the "valley" is exactly the same with the sample that was kept at room temperature (25 °C). This observation clearly shows that oregano protected effectively the corn oil sample from chemical composition changes through heating.

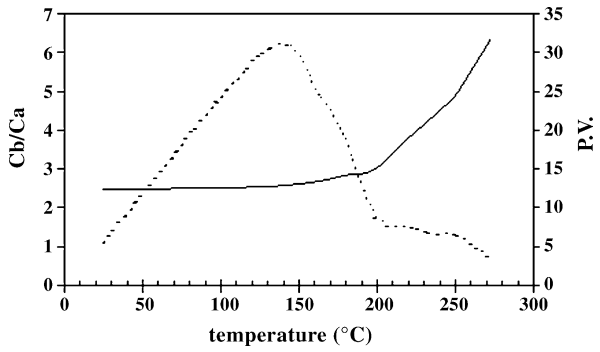

Fig. 7. Effect of different heating temperature on: (a) peroxide value (P.V.) (dashed line) and (b) the total amount of carbonyl compounds (solid line) (heating time was 30 min).

It is also interesting to follow the spectral changes in the C=O region (~1746 cm^{-1}). Here, the study shows a widening of the band (Fig. 6) for the heated samples. This observation is due to production of saturated aldehyde functional groups or other secondary oxidation products that cause an absorbance at 1728 cm^{-1}, which overlaps with the stretching vibration at 1746 cm^{-1} of the ester carbonyl functional group of the triglyc-

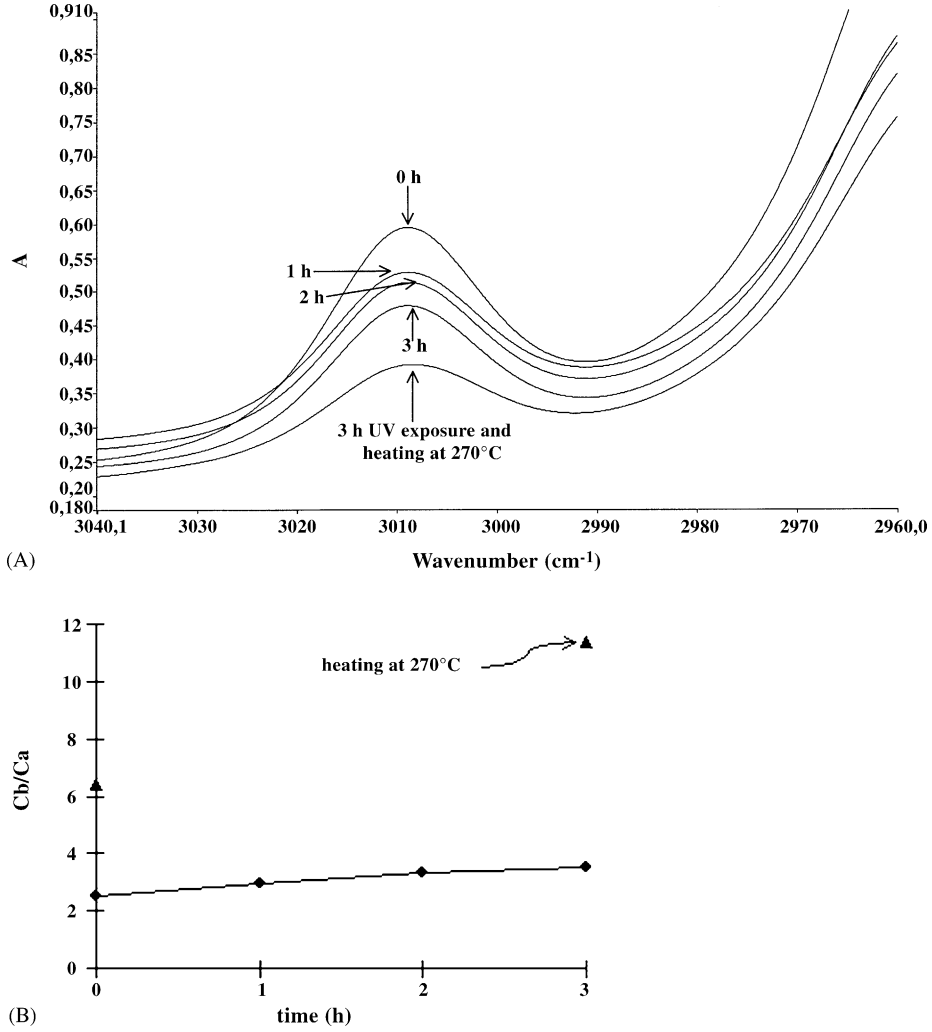

Fig. 8. (A) Spectra of corn oil samples exposed to ultraviolet radiation and heated. (B) The total amount of carbonyl compounds vs. UV exposure time.

erides. When new carbonyls are formed from initial aldehyde and ketone compounds, the maximum absorbance is in the region between 1700 and 1726 cm^{-1} resulting in a broadening of the 1743 cm^{-1} band to lower wavenumbers.

In order to quantify the aforementioned spectroscopic remarks, we used a method developed by Moya Moreno et al. [16]. The method is based on the C=O stretching frequency of aldehydes and ketones. Aldehydes show maximum absorbance at 1725 cm^{-1}, whereas ketones show a maximum at 1715 cm^{-1}. Consequently, the total amount of carbonyls formed, can be quantitatively measured by the broadening of the 1743 cm^{-1} band, i.e. by calculating the area from 1700 to 1726 cm^{-1} (A_1) and comparing it to the area from 1743 to 1840 cm^{-1} ($A_{1/2}$). The last area is attributed to the esters of the triglycerides exclusively. The calibration curve obtained from 11 standards was a linear equation $A_1/A_{1/2} = (0.0090 \pm 0.0001)(C_b/C_a) + (0.0940 \pm 0.0017)$ with a correlation index of 0.999, where C_b/C_a is the mass ratio of butanal in the oil.

Fig. 7 shows the concentrations of carbonyls expressed as % weight of butanal, for each temperature at which the sample was heated for 30 min. After 200 °C there is an exponential increase in carbonylic compounds. Study of the accuracy of the analytical method gave a 2% relative standard deviation value for seven replicates. In the same plot the peroxide value versus heating temperature is presented. It is obvious that after the decrease of the primary oxidation products (dashed line, Fig. 7), the secondary products increase (solid line, Fig. 7). The peroxide value obtained for each sample by the iodimetric method, reveals that the increase of the carbonyl compounds coincide with the decomposition of the hydroperoxides. The same behavior was observed for extra virgin olive oil in similar experiments.

3.3.2. *Effect of exposure to ultraviolet radiation*

The effect of ultraviolet radiation on corn oil samples and its synergistic effect with heating, in the oil quality were also studied. For this reason, a sample was exposed for 1, 2 and 3 h to ultraviolet radiation. The sample exposed for 3 h was also heated for 30 min at 270 °C. The frequency value of the 3009 cm^{-1} band shows a small shifting to lower values as the UV exposure time increases (Fig. 8A). This shifting is more pronounced for the sample exposed for 3 h to UV radiation and then heated at 270 °C for 30 min. This happened because the *cis*-olefinic double bonds of the different acyl groups disappeared during the oxidation process.

Moreover, after processing of the carbonyl region spectral data, a widening of the 1746 cm^{-1} band was observed. The reason for this change was explained in Section 3.3.1. Fig. 8B shows a slight increase of carbonyls for the irradiated samples. If we compare Figs. 7 and 8B we notice that a 3 h irradiation produces the same effect as heating at 220 °C (same value of C_b/C_a). On the contrary, the increase of carbonyls is significant for the sample that underwent both UV exposure and heating. Figs. 7 and 8B show the huge difference of the maximum values reached for C_b/C_a for the different treatments. In Fig. 7 the maximum value is only 6.4 while in Fig. 8B this value is almost two times higher (11.4). This fact reveals the significant impact of a possible prior UV exposure on the degradation extent of oil during heating.

4. Conclusions

Specific FT-IR spectral regions prove to be very useful for the determination of adulteration as well as for the study of the oxidation process. A band shift observed at 3009 cm^{-1} has been used to determine extra virgin olive oil adulteration with different types of vegetable oils. The present study indicates that the detection limit for oil adulteration is 9% if the adulterant is corn oil or sesame seed oil while it is lower (6%) if the adulterant is sunflower oil or soyabean oil. Furthermore, spectral changes appearing in the 3050–2800 and 1745 cm^{-1} region, after heating at elevated temperatures and/or exposure to ultraviolet radiation, aid the oxidation process monitoring. These changes are not present if an antioxidant compound such as oregano is added to the oil before heating while they are huge if the oil is also exposed to ultraviolet radiation. This methodology could be useful to evaluate the oxidative state of edible oils in a simple and fast way.

References

[1] M.D. Guillen, N. Cabo, J. Agric. Food Chem. 47 (1999) 709.
[2] M.D. Guillen, N. Cabo, J. Sci. Food Agric. 80 (2000) 2028.
[3] M.D. Guillen, N. Cabo, Food Chem. 77 (2002) 503.
[4] D. Goburdhun, S.B. Jhaumeer-Laulloo, R. Musruck, Int. J. Food Sci. Nutr. 52 (2001) 31.
[5] F.R. van de Voort, Food Res. Int. 25 (1992) 397.
[6] F.R. van de Voort, J. Sedman, T. Russin, Eur. J. Lipid Sci. Technol. 103 (2001) 815, and references therein.
[7] M.D. Guillen, N. Cabo, J. Sci. Food Agric. 75 (1997) 1, and references therein.
[8] A. Tay, R.K. Singh, S.S. Krishnan, J.P. Gore, Lebens. Wissen. Technol. 35 (2002) 99.
[9] L. Kupper, H.M. Heise, P. Lampen, A.N. Davies, P. McIntyre, Appl. Spectrosc. 55 (2001) 563.
[10] N.A. Marigheto, E.K. Kemsley, M. Defernez, R.H. Wilson, J. Am. Oil Chem. Soc. 75 (1998) 987.
[11] Y.W. Lai, E.K. Kemsley, R.H. Wilson, Food Chem. 53 (1995) 95.
[12] B.F. Ozen, L.J. Mauer, J. Agric. Food Chem. 50 (2002) 3898.
[13] B.F. Ozen, I. Weiss, L.J. Mauer, J. Agric. Food Chem. 51 (2003) 5871.
[14] T. Russin, F.R. van de Voort, J. Am. Oil Chem. Soc. 81 (2) (2004) 111.
[15] F.R. van de Voort, A.A. Ismail, J. Sedman, G. Emo, J. Am. Oil Chem. Soc. 71 (3) (1994) 24.
[16] M.C.M. Moya Moreno, D. Mendoza Olivares, F.J. Amezquita Lopez, V. Peris Martinez, F. Bosch Reig, J. Mol. Struct. 482–483 (1999) 557.
[17] Official Press of European Community, No. 1248, 8-9, 5 September 1991.
[18] National Food and Drink Code, Chapter 8, 2003, based on (i) Comission Regulation E.E.C. 2568/11.07.91 "on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis" and (ii) unpublished data of General Chemical State Laboratory.
[19] A. Tomaino, F. Cimino, V. Zimbalatti, V. Venuti, V. Sulfaro, A. De Pasquale, A. Saija, Food Chem. 89 (2005) 549.
[20] Y.B. Che Man, I. Jaswir, Food Chem. 69 (2000) 301.
[21] M.H. Gordon, L. Kourimaska, J. Sci. Food Agric. 68 (1995) 347.
[22] P. Zandi, M.H. Gordon, Food Chem. 64 (1999) 285.