

Two-dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy

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Abstract

Accelerate oxidative degradation of six vegetable oils was monitored using FTIR and FT-Raman spectroscopy. Two-dimensional correlation spectroscopy and multivariate curve resolution alternating least squares (MCR-ALS) were applied to the analysis of the data. The use of heterospectral two-dimensional correlation of FTIR and FT-Raman data allowed the use of well established band assignments to interpret less clearly assigned spectral features. With a moving window approach it was possible to obtain simplified two-dimensional correlation maps and to detect compounds evolving with different kinetic. Simultaneous analysis of the oxidation experiments of the six different oils monitored by both spectroscopic techniques was performed using MCR-ALS. Although a complete resolution of the data was not possible, the spectral changes occurring during the oxidative degradation of the oils were described with a five-component model. The two fundamentally different chemometric approaches lead to coincident results.

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1. Introduction

Lipid oxidation is a major deteriorative reaction affecting edible oils and fats and consequently of primary concern to processors and consumers. Unsaturated lipids are particularly susceptible to oxidation. The oxidation process is extremely complex, involving numerous reactions that give rise to a variety of chemical and physical changes in lipids. The most common mechanism of oxidation is a free radical chain reaction. This process is retarded by antioxidants such as tocopherols, and accelerated by prooxidants such as trace metals and heat. Primary products in lipid oxidation are allylic hydroperoxides, in which the double bonds remain but may have changed position and/or configuration from their original form. Hydroperoxides

decompose into secondary oxidation products such as aldehydes, ketones and epoxides [1].

Classical chemical parameters used to determine the extent of oxidation in edible oils are peroxide value and spectrophotometric absorption at 232 and 270 nm, for initial stages of oxidation; and anisidine and 2-thiobarbituric acid (TBA) value that measure aldehydes, oxidation products which are closely related to the development of rancidity. More sophisticated methods use gas and liquid chromatography to identify and quantify individual oxidation products [2].

It has been demonstrated that infrared spectroscopy is a useful tool to monitor the oxidation of edible oils due to the high information content of infrared spectra and the possibility to assign specific absorption bands to particular functional groups. Spectral changes in the mid-infrared region associated with oil oxidation have been described in detail and common oxidation products, such as aldehydes, alcohols and peroxides have been identified using representative reference compounds

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[3]. Furthermore, quantitative and semi-quantitative approaches have been proposed to evaluate the oxidative alteration of oils using FTIR [4–6].

In a previous work we reported the possibility of monitoring oil oxidation using FT-Raman spectroscopy [7]. Formation of aldehydes was detected and isomerization of *cis* to *trans* double bonds, as well as formation of conjugated double bond systems was observed in the C=C stretching region. The time dependent intensity changes in certain Raman bands were compared to conventional parameters used to determine the extent of oxidation in oils, such as anisidine value and K_{270} , and showed good correlation.

Raman and IR are complementary techniques. Both techniques probe vibrational transitions but the selection rules applying for Raman scattering and IR absorption are different. Thus, the relative band intensities in IR and Raman spectra differ markedly, providing complementary information. Of particular significance in the field of vegetable oils is that stretching of C=C bonds give rise to strong Raman signals, whereas C=O stretching bands are very weak. The contrary is found in infrared spectra. The combined information provided from both techniques can be thus very useful in the study of vegetable oil oxidation, where these functional moieties are implicated in the major chemical changes.

The goal of the present work is to compare the IR and Raman spectral changes taking place during thermal oxidation of vegetable oils with different fatty acid compositions and to combine the information obtained from both techniques to gain insight on the chemical changes that occur. For this, two advanced chemometric tools for the analysis of evolving systems, two-dimensional correlation spectroscopy and multivariate curve resolution-alternating least squares will be used. Evolving data sets are frequently found in analytical chemistry, when spectroscopic techniques are applied to the monitoring of dynamic processes that proceed as a function of a modulation variable. In contrast to the univariate approaches analyzing specifically predetermined peaks, which were usually applied in the study of oil oxidation with infrared and in our previous study with Raman spectroscopy, these multivariate techniques allow comprehensive analysis of the whole spectra.

Two-dimensional correlation spectroscopy (2D-CoS) performs cross-correlation analysis of a series of spectra of a system that changes with a modulation variable. It spreads the one-dimensional spectra into a second spectral dimension, thus yielding two-dimensional maps of cross-correlations of a given spectral variable with every other spectral variable in the data [8]. In the most recent formulation, the synchronous correlation map is obtained as a covariance matrix of the measured spectra and the asynchronous correlation map as product of the matrix of measured spectra and a Hilbert–Noda transform [9,10]. 2D-CoS gives a descriptive picture of the data by visualizing and enhancing correlation (synchronous map) and disrelation (asynchronous map) in the data. It enhances spectral resolution and allows the establishment of unambiguous assignments of bands. Furthermore, the use of hetero-spectral correlation analysis provides a convenient mean to investigate the relationship

between dynamic variations observed by two different spectroscopic techniques [11,12].

The goal of curve resolution methods is to determine the number of significant chemical/physical components that contribute spectroscopically to the measured data matrix and recover the pure response profiles (pure spectra and pure concentration profile) of the modeled components [13,14]. This is done by decomposing the experimentally measured data matrix into the product of two smaller matrices containing the concentration profiles and the spectra. Various curve resolution approaches have been proposed, which differ in their principles and requirements to the data structure. Multivariate curve resolution-alternating least squares is a very flexible method that allows for the inclusion of several chemical constraints and for the simultaneous analysis of multiple data sets [15,16].

2. Experimental

2.1. Instrumentation

Raman spectra were recorded with a Bruker RFS (FT)-Raman spectrometer fitted with a liquid nitrogen cooled Ge detector. The samples were illuminated by an Nd:YAG laser line at 1064 nm with a power of 500 mW using a focused laser beam. All spectra were recorded with a resolution of 4 cm^{-1} and averaged over 200 scans. Acquisition of one spectrum took 5 min. Infrared spectra were recorded with a Bruker Tensor 37 Fourier transform infrared spectrometer equipped with a horizontal DuraSamplIR II triple reflection ATR (SensIR Technologies) and a narrow-band mercury cadmium telluride (MCT) detector. All spectra were recorded at 4 cm^{-1} resolution (128 scans, apodization function: Blackman-Harris-3-term). Spectra of the clean and dry ATR crystal against air were used for the background.

2.2. Samples

The six vegetable oils, virgin olive, corn, sunflower, rapeseed, peanut and safflower, were purchased from Austrian and Spanish supermarkets. *Cis*-9, *trans*-11-linoleic acid standard was supplied by Sigma.

2.3. Oxidation and spectroscopic measurements

About 200 mL of the oils were heated at $160 (\pm 2)^\circ\text{C}$ and stirred continuously on a magnetic stirrer for 9 h. Every half an hour 4 mL were sampled, left to cool to room temperature for recording the FT-Raman and FT-IR spectra. No sample preparation was required.

2.4. Data evaluation

For all data evaluation the experimental data obtained for each oil are assembled in matrix **D**, which represents an $m \times n$ matrix with spectra (Raman or infrared) aligned in rows, thus yielding a time $\times$ wavenumber matrix.

2.5. Two-dimensional correlation spectroscopy

The calculation of two-dimensional correlation maps was performed on the mean normalized, mean-centered spectral data. Synchronous and asynchronous correlation maps were obtained with the calculation based on linear algebra [10]. In synchronous maps, autopeaks (always positive) indicate changes at this wavenumber during the process under study. Furthermore, cross peaks develop between bands that change simultaneous or coincidentally during the process. Positive cross-peaks are positive if both bands are increasing or decreasing. Negative cross-peaks indicate that one of the bands is increasing while the other is decreasing. In asynchronous correlation maps spectral features that change out of phase are revealed. The sign of the asynchronous cross-peaks can be useful to establish the order of events taking place.

Moving window correlation spectroscopy was carried out calculating synchronous and asynchronous correlation maps on small data matrices that were obtained by partitioning **D** into sets of submatrices [17]. The moving window concept refers to the selection of arbitrarily sized submatrices that start from the first measurement and are then shifted by one place all the way to the last measurement. For *N* data and a moving window (submatrix) size of *n*, there will be total of *N* − *n* + 1 submatrices. A window size of four spectra was employed, resulting in 16 windows. Correlation maps were calculated with Matlab [18] programs on the basis of the freely available 2D-CoS Toolbox by Berry and Ozaki [19].

2.6. Multivariate curve resolution-alternating least squares (MCR-ALS)

The goal of MCR-ALS is the recovery of the concentration profiles and the pure spectra of each the component of the system, according to:

$$\mathbf{D} = \mathbf{C}\mathbf{S}^T + \mathbf{E}$$

where **C** is a matrix of pure concentration profiles, **S**^T is the matrix of corresponding pure spectra and **E** the matrix of residuals, not explained by the modeled components. MCR-ALS solves this equation iteratively by an alternating least squares algorithm that calculates concentration **C** and pure spectra matrices **S**^T optimally fitting experimental data matrix **D** using the proposed number of components and minimizing the error matrix **E**. The number of components or contributions to be considered in the mathematical decomposition was determined by singular value decomposition (SVD) and principal component analysis (PCA). The iterative optimization requires initial estimates of spectra or concentration profiles that were obtained using SIMPLS-to-use interactive self-modeling mixture analysis (SIMPLISMA) [20]. This algorithm is based on the use of real variables and finds the purest or more representative contributions to the data matrix. It assumes the existence of a wavelength where the selectivity of a given component is maximized, i.e. that experiences substantial intensity contribution from only one of the components in the mixture. Consequently the intensities of these pure variables can be related to concen-

tration profiles. Constrains applied during the ALS optimization were non-negativity for concentration profiles and for infrared and Raman spectra and unimodality for concentration profiles. Theory and application of MCR-ALS have been described in detail elsewhere [14,15]. The freely available program (Matlab code) by A. de Juan and R. Tauler was used for MCR-ALS analysis [21].

3. Results and discussion

3.1. Infrared spectra of edible oils during oxidation

Fig. 1 shows the changes in the infrared spectra of corn oil during nine hours of oxidation in the regions 600–1800 cm^{−1} and 2750–3600 cm^{−1}. The major bands in the non-oxidized oil can be attributed to the following vibrations [22]: 710 cm^{−1} (CH wag in *cis* double bond), 1160 cm^{−1} (CH₂ wag), 1377 cm^{−1} (sym HCH bending), 1462 cm^{−1} (CH₂ scis), 1743 (ester stretching ν(C=O)) 2854 cm^{−1} (CH₂ symmetric stretch ν(C–H)), 2924 cm^{−1} (CH₂ asymmetric stretch ν(C–H)), 3008 cm^{−1} (symmetric stretch in *cis* double bond ν(=C–H)).

During the oxidation process the band at 710 cm^{−1} decreases, which is in accordance with the loss of *cis* double bonds. The increase of two bands at 970 and 987 cm^{−1} can be assigned to formation of *trans* double bonds. In the region between 1000–1400 cm^{−1} a general low increase in absorption can be observed, which can not be attributed to certain bands. Only the bands at 1160 and 1462 cm^{−1} remain nearly unaltered. Another increase in absorbance can be observed in the region 1660–1735 cm^{−1}, which is due to carbonyl compounds formed during oxidation. A decrease in absorbance is furthermore seen in the band at 1743 cm^{−1}, which is more difficult to interpret. The triglyceride ester linkage is one of the most stable bonds in an oil system. Extensive hydrolysis only happens at temperatures higher than 185 °C and in the presence of moisture. Thus, in the conditions of this study cleavage of the ester link is not expected. Furthermore, in the high wavenumber region there are no bands around 3300 cm^{−1} indicating the presence of free fatty acids. A similar observation was made by van de Voort, who attributed

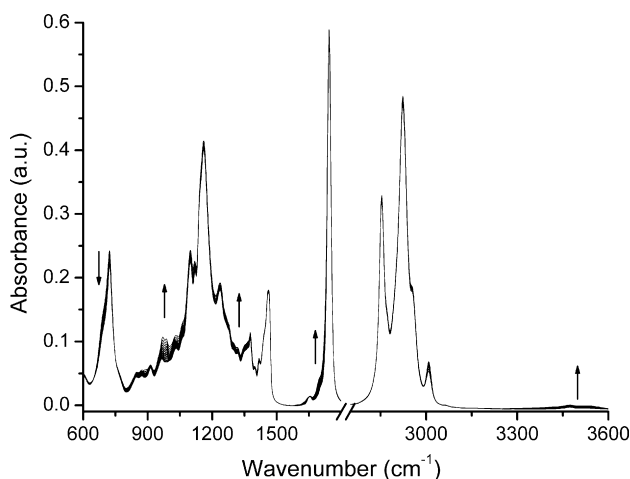


Fig. 1. IR spectra of corn oil during 9 h of oxidation.

the decrease of the ester band to a dilution effect due to oxygen uptake [3]. The two bands at 2854 and 2924 cm^{-1} remain nearly unaltered. A decrease is observed at the band 3008 cm^{-1} , which derives from the decrease in *cis* double bonds and finally, a very weak increase in absorbance is observed around 3550 cm^{-1} , which can be assigned to the OH stretching vibration in alcohols, which are formed during oxidation. Generally, the spectral region above 1800 cm^{-1} is not very informative with the most important change observed at 3008 cm^{-1} . The same information is obtained at 710 cm^{-1} , it was therefore decided to solely use the region between 600 and 1800 cm^{-1} for the two-dimensional infrared correlation spectroscopy.

3.2. Two-dimensional infrared correlation spectroscopy

Synchronous and asynchronous correlation maps were very similar for the different oils. In Fig. 2 the synchronous and asynchronous correlation maps of corn oil as example are presented. In Table 1 a summary of the more relevant peaks for the different oils is presented. The synchronous correlation maps provide information about the major changes taking place in the infrared spectrum of the oils as oxidation proceeds. The formation of saturated carbonyl compounds that absorb around 1727 cm^{-1} , alcohols in the region of 1110 cm^{-1} and *trans* double bonds absorbing at 970 cm^{-1} is observed in all oils. The decrease of the band at 710 cm^{-1} as a consequence of the lost of *cis* double bond due to isomerization is also seen in all oils.

In the synchronous correlation maps of olive oil none of the correlation peaks involving bands at 1690 and 987 cm^{-1} are observed. The same happens with the band at 987 cm^{-1} for peanut and rapeseed oils. These two bands must correspond to oxidation products, which are formed from polyunsaturated fatty acids, such as conjugated aldehydes and conjugated double bond systems. This is reason why these peaks are of lower intensity or are even absent in oils with lower amount of linoleic and linolenic acid and especially in olive oil (see Table 2).

With the help of the asynchronous map more resolution of some bands can be achieved. Thus, different asynchronous

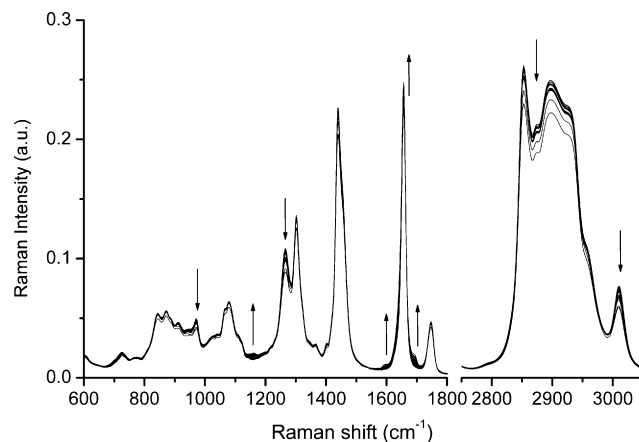


Fig. 3. FT-Raman spectra of corn oil during 9 h of oxidation.

peaks in the region around 1700 cm^{-1} can be identified. These peaks can be attributed to the different carbonyl compounds, which are formed during oxidation, namely saturated aldehydes (1732 cm^{-1}), saturated ketones (1710 cm^{-1}) and unsaturated aldehydes (1690 cm^{-1}). The band at 1640 cm^{-1} probably corresponds to the C=C stretching absorption of unsaturated carbonyl compounds, which exhibits exceptional infrared intensity due to conjugation with the aldehyde group. The absence of an asynchronous cross peak between 1640 and 1690 cm^{-1} confirms this assumption. The band at 1747 cm^{-1} gives rise to strong asynchronous peaks with all the bands changing during oxidation, which confirms the different origin of the changes at this position. The strong asynchronous peaks developed between 987 and 970 cm^{-1} indicate clearly that these two bands come from different compounds formed during oxidation at different rate. Especially noticeable is the asynchronous peak between 987 and 710 cm^{-1} , which is very strong and accompanied by a peak at 950 cm^{-1} . The bands at 987 and 950 cm^{-1} can be assigned to the CH wag vibration in conjugated *trans*, *trans* and *cis*, *trans* double bond systems. The band at 970 cm^{-1} corresponds to CH wag in isolated *trans* double bonds and contrary to the band at 987 cm^{-1} shows no asynchronous peak with the band at 710 cm^{-1} (CH

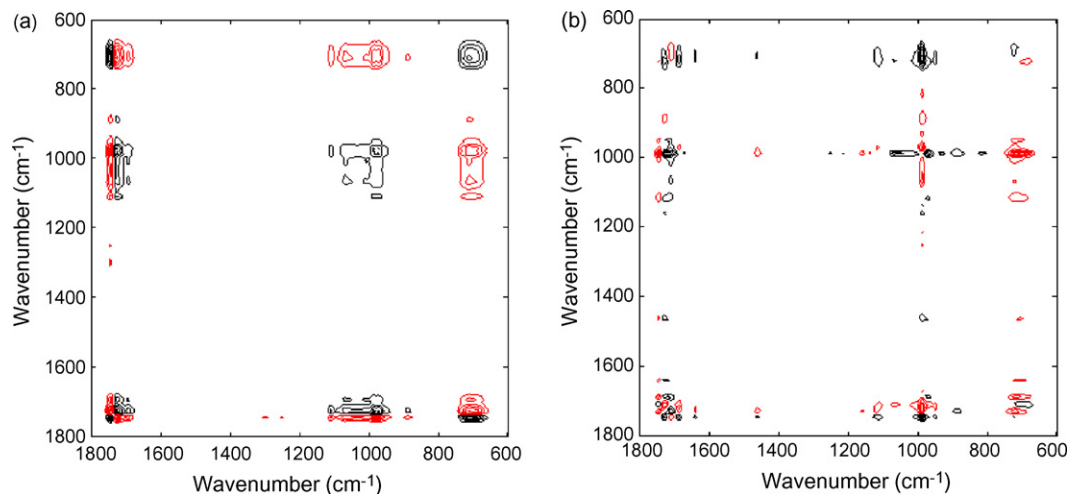


Fig. 2. (a) synchronous 2D IR correlation map and (b) asynchronous 2D IR correlation map (corn oil) Positive peaks in black, negative peaks in red.

Table 1
Summary of peaks in 2D IR correlation maps

Synchronous maps		
Autopeaks	1747	All oils
	1727	All oils
	1690	Peanut, rapeseed, corn, sunflower, safflower
	1060	Corn, sunflower, safflower
	987	Corn, sunflower, safflower
	970	All oils
	710	All oils
Positive cross-peaks	1747–710	All oils
	1727–1690	Peanut, rapeseed, corn, sunflower, safflower
	1727–1110	All oils
	1727–1060	All oils
	1727–987	Corn, sunflower, safflower
	1727–970	All oils
Negative Cross-peaks	1747–1727	All oils
	1747–1690	Peanut, rapeseed, corn, sunflower, safflower
	1747–1100	All oils
	1747–1060	All oils
	1747–987	Corn, sunflower, safflower
	1747–970	All oils
	1727–710	All oils
	1695–710	Corn, sunflower, safflower
	1100–710	Peanut, rapeseed, corn, sunflower, safflower
	1060–710	All oils
	987–710	Corn, sunflower, safflower
	970–710	All oils
Asynchronous maps		
Asynchronous cross-peaks	1747–1732	All oils
	1747–1710	All oils
	1747–1690	All oils
	1747–1640	Rapeseed, corn, sunflower, safflower
	1747–987	All oils
	1747–950	Peanut, rapeseed, corn, sunflower, safflower
	1747–710	All oils (weak)
	1732–987	All oils
	1710–987	All oils
	1710–950	Peanut, rapeseed, corn, sunflower, safflower
	1690–970	Corn, sunflower
	987–970	Peanut, rapeseed, corn, sunflower, safflower
	987–710	Peanut, rapeseed, corn, sunflower, safflower

wag of *cis* double bonds). It is interesting to note that the asynchronous peak between 1747 and 1695 cm^{-1} also appears in olive oil. This is due to the major sensitivity of the asynchronous correlation map to detect small changes when they occur at different rate than major changes. The same happens with the band at 987 cm^{-1} , which is observed in the asynchronous map in all the oils.

3.3. Raman spectra of edible oils during oxidation

Fig. 3 shows the changes in the Raman spectra of corn oil during nine hours of oxidation in the regions 600–1800 cm^{-1} and 2750–3050 cm^{-1} . The major bands in the non-oxidized oil can be found at 1080 cm^{-1} ($\nu(\text{CC})$, $-(\text{CH}_2)_n$), 1267 cm^{-1} (symmetric rock in *cis* double bond $\delta(\text{=C-H})$), 1302 cm^{-1} (in-phase

Table 2
Fatty acid compositions and iodine values of the six vegetable oils used in this study

	Olive	Peanut	Rapeseed	Corn	Sunflower	Safflower
C 16:0 + C 18:0	12.7	11.3	6.0	12.0	10.2	6.6
C 18:1	81.2	50.2	64.4	31.9	30.4	19.5
C 18:2	4.8	33.2	19.5	54.7	59.1	70.7
C 18:3	0.6	2.1	9.8	1.0	0.2	0.9
IV	80	99	110	128	123	166

C 16:0 = palmitic acid, C 18:0 = stearic acid, C 18:1 = oleic acid, C 18:2 = linoleic acid, C 18:3 = linolenic acid; values for acids reflect percentage of sum, IV = iodine value.

methylene twist), 1442 cm^{-1} (CH_2 scis), 1655 cm^{-1} (*cis* double bond stretching $\nu(\text{C}=\text{C})$), and 1747 cm^{-1} (ester stretching $\nu(\text{C}=\text{O})$). The region between 2850 and 2980 cm^{-1} is characteristic of the symmetric and asymmetric C–H stretching vibrations of methyl and methylene groups. At about 3007 cm^{-1} the symmetric $=\text{C}-\text{H}$ stretching vibration can be found. [23,24] During oxidation the major changes are the decrease of the bands at 1267 cm^{-1} and 3007 cm^{-1} due to the loss of *cis* double bonds and formation of a band at about 1680 cm^{-1} mostly due to carbonylic compounds. Furthermore, changes in the band at 1655 cm^{-1} can be observed. They include increases, decreases and shifts, which are dependent on the oil type and not easy to interpret.

3.4. Two-dimensional Raman correlation spectroscopy

A summary of the most significant correlation peaks obtained with 2D Raman correlation is presented in Table 3. In contrast to

the synchronous and asynchronous correlation maps in infrared, in Raman they show distinct patterns for the different oils. This is mostly due to the behavior of the band at around 1655 cm^{-1} . Nevertheless, in all oils a strong autopeak develops at around $1650\text{--}1655\text{ cm}^{-1}$ and a more or less strong one at 1267 cm^{-1} . Always positive cross-peaks are observed between the bands at 1267 cm^{-1} and the band at 970 cm^{-1} , bands which are both decreasing as oxidation proceeds. This cross-peak is absent in the asynchronous maps, which suggests the assignment of the band at 970 cm^{-1} to *cis* double bond vibrations despite this band has been assigned to *trans* double bonds in previous works [25,26], probably due to the existence of a band at this position in the infrared spectrum. Furthermore, a strong peak is always observed between the bands at 1655 and 1267 cm^{-1} in the asynchronous maps, which indicates that in the band at 1655 cm^{-1} other changes than just the loss of *cis* double bonds occur.

The synchronous and asynchronous correlation maps in the region $1600\text{--}1720\text{ cm}^{-1}$ (the region showing the most signifi-

Table 3
Summary of peaks in 2D Raman correlation maps

Synchronous maps		
Autopeaks	1690 1680 1672 1655 1648 1640 1267 970	Peanut, sunflower Rapeseed Peanut, sunflower Olive, peanut, rapeseed Corn, sunflower, safflower Peanut, rapeseed All oils All oils
Positive cross-peaks	1690–1672 1690–1648 1690–1640 1672–1648 1655–1267 1648–1180 1267–970	Peanut Corn, sunflower, safflower Peanut, rapeseed Corn, sunflower, safflower Olive, peanut Corn, sunflower, safflower All oils
Negative Cross-peaks	1725–1655 1690–1655 1690–1267 1690–970 1672–1655 1672–1267 1655–1630 1648–1267 1648–970 1640–1267 1640–970	Olive Olive, peanut, rapeseed Peanut, rapeseed, corn, sunflower, safflower Peanut Olive, peanut, rapeseed Peanut, rapeseed, corn, sunflower, safflower Olive, peanut, rapeseed Corn, sunflower, safflower Corn, sunflower, safflower Peanut, rapeseed Peanut, rapeseed
Asynchronous maps		
Asynchronous cross-peaks	1695–1655 1695–1640 1672–1655 1672–1640 1658–1650 1655–1640 1655–1525 1655–1267 1655–970 1648–1638 1635–1620	Olive, peanut, rapeseed Rapeseed, corn Olive, peanut, rapeseed Rapeseed, corn Corn, sunflower Olive, peanut, rapeseed Olive All oils Olive, peanut, rapeseed Corn, sunflower, safflower Corn

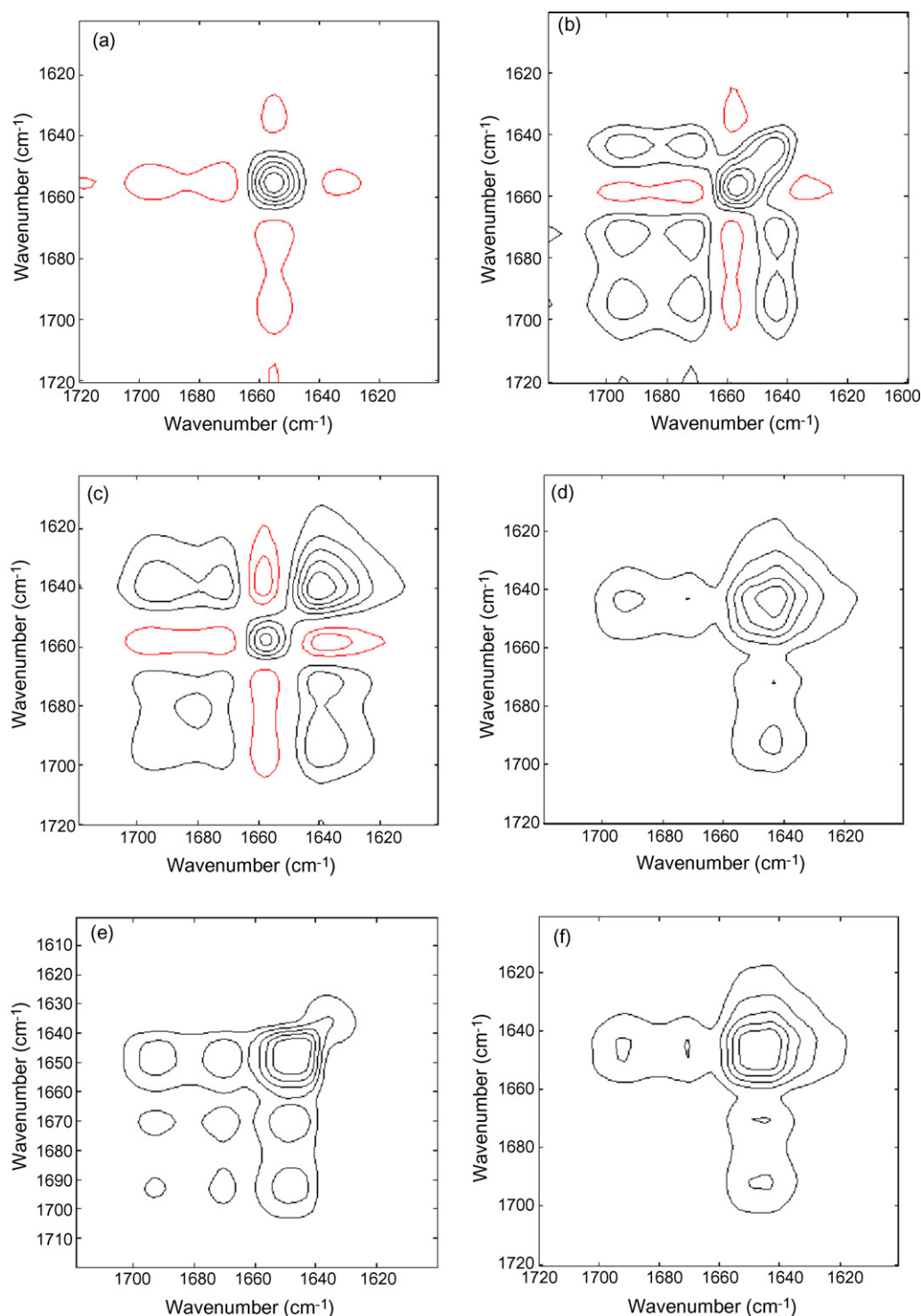


Fig. 4. Synchronous 2D FT-Raman correlation maps of the different oils: (a) olive, (b) peanut, (c) rapeseed, (d) corn, (e) sunflower and (f) safflower. Positive peaks in black, negative peaks in red.

cant features for all oils) are presented in Figs. 4(a–f) and 5(a–f), respectively.

Olive oil shows the simplest pattern. The observed peaks reflect the formation of saturated aldehydes (1725 cm^{-1}), α,β -unsaturated aldehydes (1695 cm^{-1}) and isolated *trans* double bonds (1672 cm^{-1}) at different rates. The decrease at 1655 cm^{-1} corresponds to the loss of *cis* double bonds and strongly overlaps

with the increase of another band at 1640 cm^{-1} which can be assigned to the C=C stretching in conjugated double bonds. This band appears in the synchronous map shifted to 1635 cm^{-1} due to the decrease of the band at 1655 cm^{-1} (the peak position in correlation maps does not necessarily mean that a band is located at that place [27]). Additionally, in the asynchronous correlation map a peak between the bands at 1655 and 1525 cm^{-1} , which

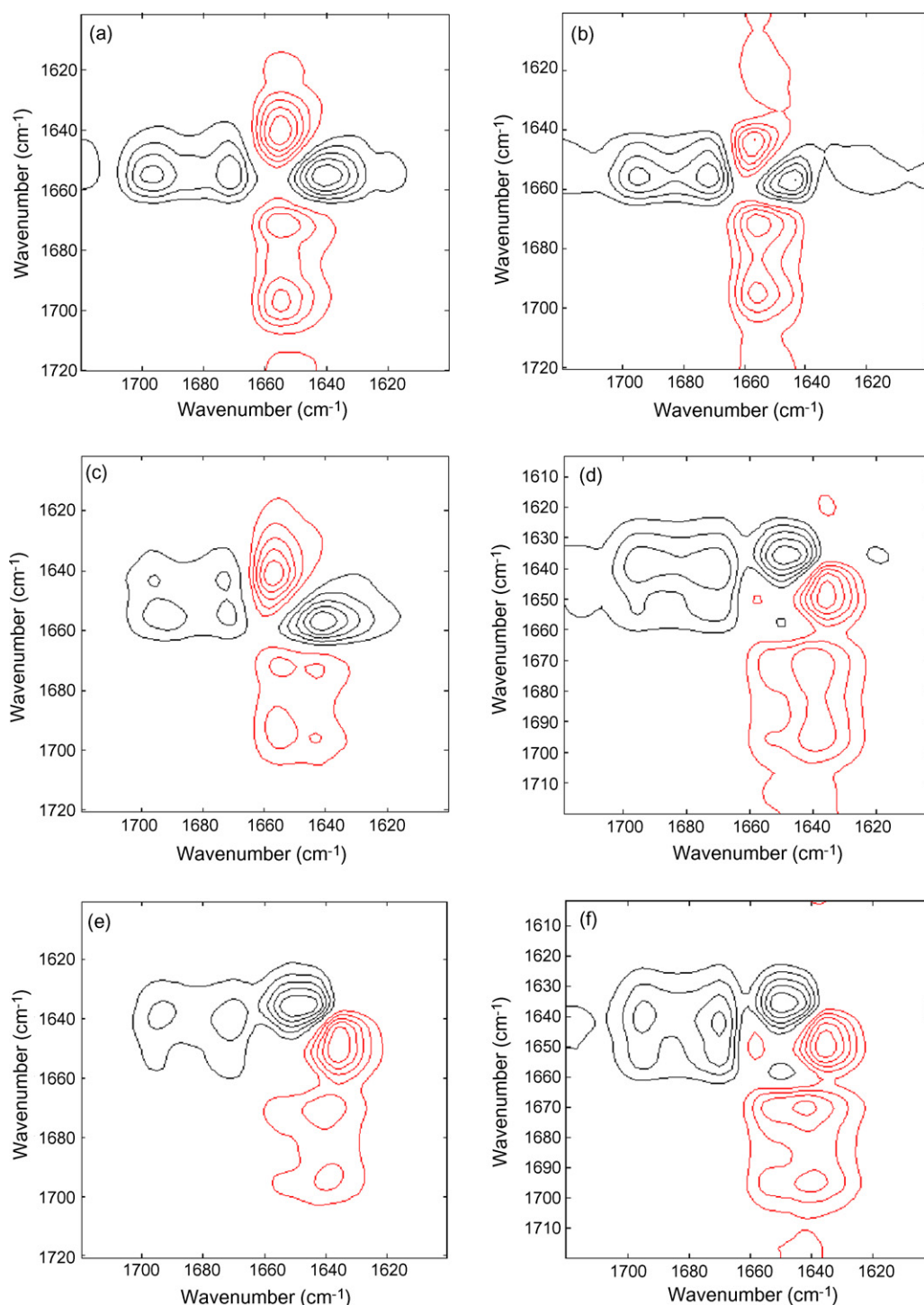


Fig. 5. Asynchronous 2D FT-Raman correlation maps of the different oils: (a) olive, (b) peanut, (c) rapeseed, (d) corn, (e) sunflower and (f) safflower. Positive peaks in black, negative peaks in red.

is unique for olive oil, develops. The correspondent autopeak at 1525 was not observed because of the small magnitude of this band, which corresponds to the C=C vibration of highly conjugated systems, like carotenoids. These compounds are natural antioxidants present in virgin olive oil, but not in refined oils. They disappear in the initial phase of the oxidation, following a completely different kinetic, which is responsible for the development of the asynchronous peak.

Peanut and rapeseed oil show similar patterns in the correlation maps. The synchronous maps are similar to olive oil but additional features can be seen. A strong autopeak at 1640 cm^{-1} appears. This band was identified in olive oil only with the help of the asynchronous map. In these oils the formation of conjugated double bonds systems occurs to a greater extent due to the higher content of polyunsaturated fatty acids. The asynchronous maps are similar to the map of olive oil, but the intensities are higher.

Corn, safflower and sunflower oil show only positive peaks in the synchronous map of this region, corresponding to the general increase also observable in the one-dimensional spectra. Thus, the formation of compounds giving rise to strong Raman signals that overlap with the decreasing band of *cis* double bonds at 1658 cm^{-1} is the more remarkable feature in these three oils.

The different appearance of the correlation maps in the oils must be due to different species with bands in this region that are appearing and disappearing during oxidation. The different kinetic and extent of formation and decomposition of these species in the different types of oil lead to the complex patterns observed. It should be mentioned that the three different patterns observed correspond to the different fatty acid composition of the oils and their iodine values. Corn, safflower and sunflower oil are rich in linoleic acid, whereas olive oil contains only five percent of polyunsaturated fatty acids. Peanut and rapeseed oil show high content in oleic acid, but also contain linoleic and linolenic fatty acids in considerable amounts (see Table 2).

3.5. Heterospectral two-dimensional infrared and Raman spectroscopy

Two-dimensional correlation spectroscopy applied to the Raman (R) and infrared (I) spectra identified in both cases peak positions, which can be related to oxidation products. Both techniques are complementary, thus the construction of heterospectral correlation maps can provide additional information about the relationships among the bands and help in the assignment of some bands. The heterospectral synchronous maps showed correlation peaks between all the bands changing with oxidation previously described. The comparison of synchronous and asynchronous correlation maps provided the following findings: (a) no asynchronous peak develops between 1267 cm^{-1} (R) and 710 cm^{-1} (I). On the contrary, these two bands give rise to a strong synchronous peak. Both bands correspond to vibrations of *cis* olefins and decrease with oxidation following the same kinetics. (b) The same happens between 970 cm^{-1} (R) and 710 cm^{-1} (I), which corroborates our assignment of the Raman band at 970 cm^{-1} to *cis* double bonds. (c) strong asynchronous peaks develop between 1640 cm^{-1} (R) and 1727 cm^{-1} (I) and 970 cm^{-1} (I). The band at 1640 cm^{-1} (R), which has been assigned to C=C stretching in conjugated systems, changes with different kinetics than the C=O stretching in saturated aldehydes (1727 cm^{-1} (I)) and –CH wag in *trans* isolated double bonds (970 cm^{-1} (I)). To further investigate the kinetics of formation of these compounds, the recently reported combination of the moving window concept with two-dimensional spectroscopy, to facilitate the analysis of complex data sets [17,28], is applied to the Raman and Infrared hetero-spectral two-dimensional correlation spectroscopy.

3.6. Moving window hetero-spectral two-dimensional correlation spectroscopy

The combination of the moving window concept with two-dimensional correlation allows one to split a large data matrix into smaller and simpler subsets and to analyze them instead

of computing overall correlation. Too complex data are difficult to analyze by means of 2D methodology because the shape of out-coming maps is due to several causes that cannot be unambiguously separated. The most straightforward way to alleviate the problem of data complexity is to analyze simpler data, that is, to analyze windows. The results of the window analysis, though seemingly numerous, are easy to compile and to extract succinct information from. Furthermore, in this study using the hetero-spectral correlation approach, the knowledge obtained from the infrared spectral changes will help in the interpretation of the less clear spectral features observed in Raman spectra. Although the hetero-spectral correlation was performed in the whole spectral range, here attention is focused on the spectral regions $1600\text{--}1740\text{ cm}^{-1}$ for Raman spectroscopy and $1670\text{--}1750$ and $900\text{--}1150\text{ cm}^{-1}$ for infrared spectroscopy where the most significant features are found and changes in the shape of the correlation maps for successive windows were observed. In Fig. 6 the synchronous maps of different windows of corn oil are shown as an example. The intensity of the correlations did not rise sharply at any moment but the shape of the maps changed. The main changes can be summarized as follows: (a) for Raman: first a positive peak at 1650 cm^{-1} rises and then negative peak at 1658 cm^{-1} develops, (b) for infrared: the peak at 970 increases until it dominates the map in this region and finally the peak at 987 disappears. These changes occur at different windows in the different oils (see Table 4) and can be interpreted as follows: at the first stages of oxidation α,β unsaturated aldehydes and $\alpha,\beta,\gamma,\delta$ conjugated unsaturated aldehydes are formed to more or less extent in the different oils. They are characterized by Raman signals around 1690 and 1640 cm^{-1} and infrared absorptions around 1690 and 974 (*trans* α,β unsaturated) and 987 (*trans*, *trans* conjugated dienals). The infrared band at 987 , which dominates at the beginning, disappears at the end of the oxidation process, indicating that conjugated double bonds are formed predominantly at early stages of oxidation. Olive oil shows noisy correlation maps, especially in the first period of the oxidation process (up to window 7, which corresponds to an oxidation time of 4.5 h). The correlations have lower intensities because in olive oil less amount of oxidation compounds are formed due to the higher stability of oleic acid against oxidation and the lower amount of polyunsaturated fatty acids [29]. In corn, safflower and sunflower oil an increasing Raman band at 1650 cm^{-1} develops at windows 6–7 (4.0–4.5 h) and at window 4 (3 h) in peanut oil. This band, which is absent in olive and rapeseed oil, could only be identified for corn, safflower and sunflower in the asynchronous two-dimensional correlation maps without the moving window approach. With this it has been identified also in peanut oil. In our previous study of oxidation of oils with Raman spectroscopy we used spectral subtraction to visualize the data and the formation of a compound (or group of compounds) giving rise to a strong Raman signal at around 1653 cm^{-1} was suggested. This was the only plausible explanation of the apparent increase in the *cis* C=C stretching vibration, even though the corresponding *cis* =CH bands at 1267 and 3007 cm^{-1} decreased. For this study infrared and Raman spectra of *cis*-9, *trans*-11-linoleic acid were additionally recorded. This compound also called rumenic acid is the more common form among the con-

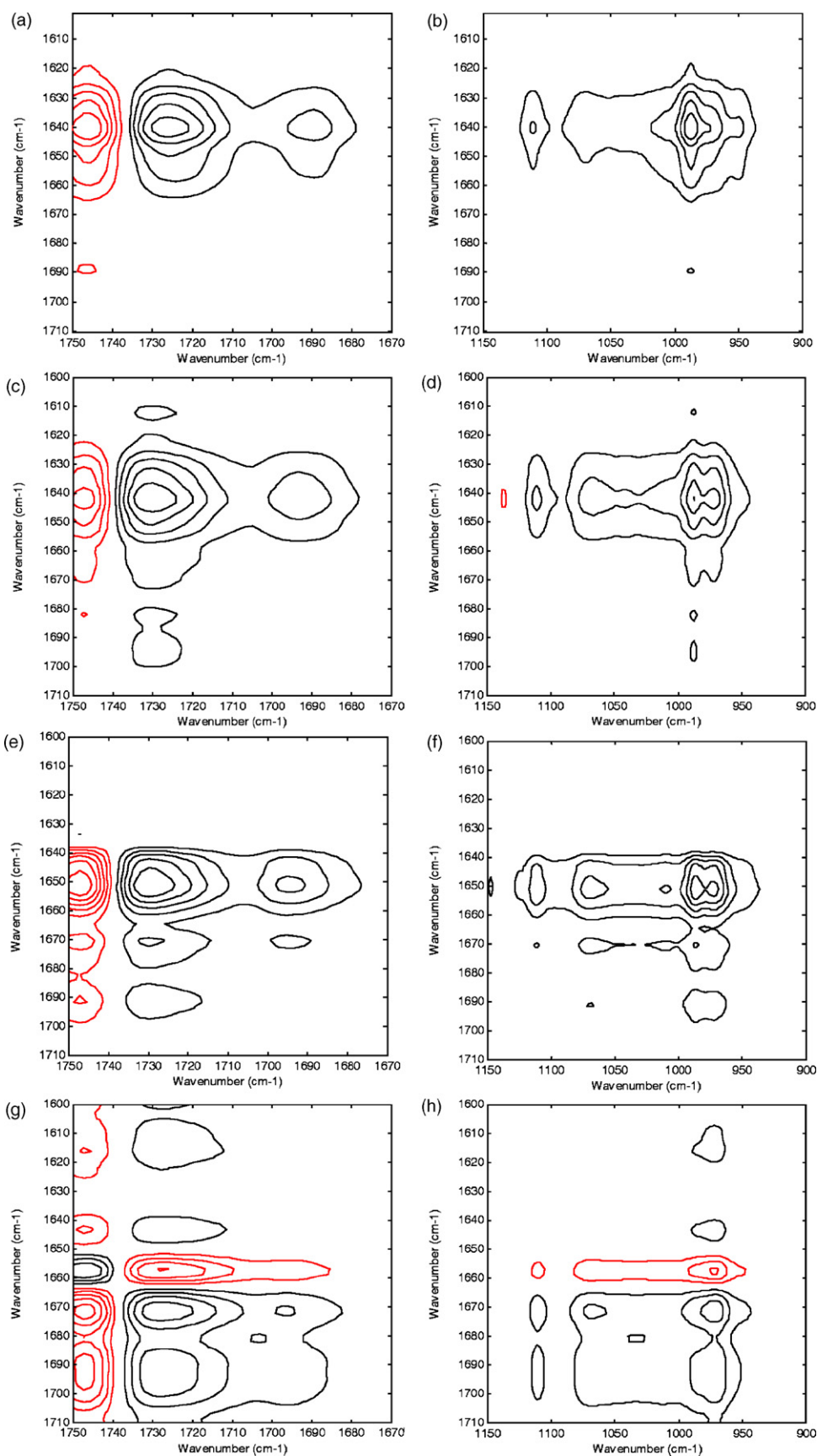


Fig. 6. Synchronous 2D IR-FT-Raman correlation maps of corn oil oxidation obtained with the moving window approach. (a), (b) Window 1, (c), (d) window 5, (e), (f) window 8 and (g), (h) window 16.

Table 4
Results of moving window hetero-spectral 2D correlation spectroscopy

	Infrared			Raman		
	987 cm ⁻¹ dominates	987 and 970 cm ⁻¹ same intensity	987 cm ⁻¹ disappears	1640 cm ⁻¹ dominates	1650 cm ⁻¹ appears	1658 cm ⁻¹ appears negative
Corn	1–6	7–16	16	1–6	7	15
Safflower	1–5	6–16	16	1–5	6	15
Sunflower	1–4	5–16	15	1–6	7	16
Peanut	1–2	3–16	10	1–3	4	11
Rapeseed	1–2	3–16	9	1–16	–	8
olive	–	1–16 ^a	–	1–16	–	2

Windows of the major changes.

^a Only 970 is visible.

jugated linoleic isomers. It shows two infrared bands at 982 and 945 cm⁻¹ that are specific for *cis*, *trans* conjugated diene groups [30], and a strong Raman band at 1653 cm⁻¹ (recently described in literature [31]), that coincides with the increasing Raman band observed during oil oxidation. Thus, it is possible to detect this compound during oil oxidation with Raman spectroscopy. The moving window hetero-spectral two-dimensional correlation spectroscopy approach furthermore allows to establish the period in the oxidation process when the formation of this compound is responsible for the major changes in the C=C stretching region. This period lasts from 4.0–4.5 to 8.0–8.5 h oxidation time for corn, safflower and sunflower and from 3.0 to 6.0 h for peanut oil, and corresponds approximately to the period in which the 987 and 970 cm⁻¹ bands of the infrared spectra have similar correlation intensities. At the moment when this compound is not formed anymore the decrease in *cis* double bonds becomes readily observable in the band at 1658 cm⁻¹. At the end of the observed period Raman bands at 1640 and 1670 cm⁻¹ are still observable. They correspond to the C=C stretching in α,β unsaturated aldehydes and *trans* isolated double bonds, respectively.

In sunflower oil an additional feature was observed. A negative peak at 1635 cm⁻¹ appears at window 8 (corresponding to 5 h of oxidation time). In the correlation maps without the moving window approach asynchronous peaks at this wavenumber were observed indicating the different origin of this band. In sunflower oil with the moving window approach, the appearance of this negative peak clearly shows the decomposition of a compound after a certain oxidation period.

3.7. Multivariate curve resolution alternating least squares (MCR-ALS)

MCR-ALS of data acquired with different spectroscopic techniques applied simultaneously to the same experiment has been proven to be a very powerful tool for the resolution of complex systems. In these situations, the different sets of measurements collected are simultaneously analyzed in a row-wise augmented data matrix [32,33]. In this study, a simultaneous analysis of IR and Raman data was performed for each oil oxidation experiment. In this way, the IR spectral information (very sensitive to vibrations involving carbonyl groups and *trans* double bonds) was combined with the Raman information (very sensitive to C=C double bonds and conjugation). Each of these

matrices was a row-wise augmented matrix containing the data obtained with IR and Raman spectroscopy, in the ranges 600–1800 cm⁻¹. Furthermore, experiments of oxidation of the different oils were analyzed simultaneously to improve the resolution of this complex system. With this approach more reliable solutions can be obtained than with single experiments [34,35], eventually removing rotational ambiguities and rank deficiency problems. Although the oils differ in composition, it has been already demonstrated by 2DCoS analyses that compounds of

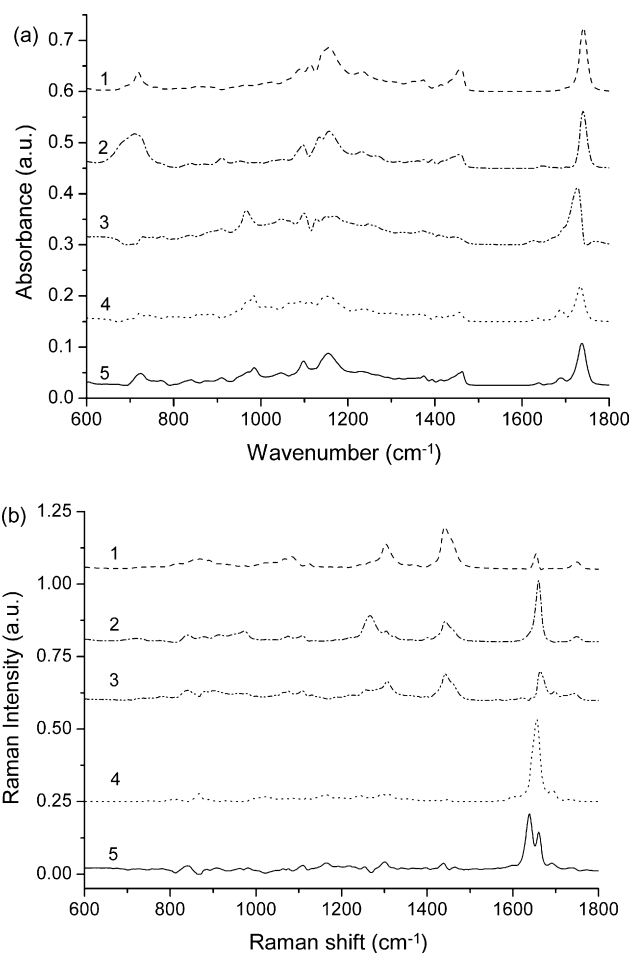


Fig. 7. MCR-ALS results from oil oxidation experiments: (a) pure infrared spectra, (b) pure Raman spectra. Component 1 (dash), Component 2 (dash dot) Component 3 (dash dot dot) Component 4 (dot) Component 5 (solid).

the same type are found during the oxidation experiments. Differences are found in the kinetic of the process and the amount of oxidation products formed. The results of the analysis of single oil oxidation experiments are not given here for the sake of brevity. Nevertheless, these results will be compared with those obtained from the column-wise augmented arrangement of the six matrices corresponding to the different oils and particularities of the different oils will be highlighted. SVD and PCA of this large array showed the presence of five components. Initial estimates for the concentration profiles were obtained using SIMPLISMA. An alternating least squares algorithm was used to obtain the matrix of pure species spectra and the matrix of species concentrations, which best fit the experimental data matrix. During optimization nonnegativity of both spectra and concentration profiles, and unimodality of concentration profiles were applied.

Considering the complexity of the oil matrix and the multitude of reaction products that are formed during oxidative degradation, it is not possible to completely resolve the spectral data into pure chemical species contributions. Consequently the obtained components reflect groups of chemical species, which evolve in a similar way during the oxidation. In Fig. 7 the pure spectra and in Fig. 8 the concentration profiles obtained are presented.

Component 1 remains almost constant during the oxidation period or decreases slightly in all the oils. The spectrum of this component resembles the spectra of vegetable oils without the bands characteristic of unsaturation. Thus, spectral features that remain unaltered during the oxidation process dominate the spectrum of this component. The isolation of this component is achieved only with the column-wise augmentation approach. If the oils are analyzed individually the constant spectral features appear mixed in all the components. Despite this component does not represent a real chemical species the isolation of the unchanged spectral features simplifies the pure spectra of the rest of components and thus facilitates the interpretation of the spectral features. Component 2 decreases during the oxidation period. The more noticeable bands in the pure spectrum of this component are characteristic of *cis* double bonds: 710 cm^{-1} in IR and 970 , 1265 and 1655 cm^{-1} in Raman spectrum. In the non-oxidized oils the concentration of this component is a measurement of unsaturation and shows good linear correlation with the iodine value of the oil ($R^2=0.883$) and an excellent correlation with the total unsaturation calculated from the GC data ($R^2=0.997$). Thus, the concentration profiles reflect the loss in double bonds as oxidation proceeds and a quantitative estimation of the total unsaturation at any time during the oxidation process becomes easily accessible. It is important to note that this quantitative information is only available with the column-wise augmentation approach. The other three components show concentration profiles that increase during the oxidation process and consequently correspond to oxidation products. Of special interest are the spectral features of these components in the C=C stretching region of the Raman spectra and in the IR regions $920\text{--}1200\text{ cm}^{-1}$ and $1600\text{--}1760\text{ cm}^{-1}$. The spectral features of these three components were also observed in the individual analyses performed for each of the oils but they appeared mixed

due to rotational ambiguity. In olive and peanut oil only two components corresponding to oxidation products were clearly found although they shared spectral features corresponding to all the components found in the combined analysis. In the individual analysis the resolution was poorer due to the low amount of oxidation products formed in these oils. This is in agreement with 2D CoS results, where with the help of sensitivity of the asynchronous maps all spectral features were also detected for these oils.

Component 3 has a similar concentration profile in all the studied oils. It increases very slowly or is even constant in the first stages of oxidation and then starts to increase faster. The more remarkable spectral features are the Raman band at 1663 cm^{-1} (with a shoulder at 1670 cm^{-1}) and the IR band at 970 cm^{-1} both characteristic of isolated *trans* double bonds; and the IR band at 1730 cm^{-1} which corresponds to saturated carbonylic compounds.

Components 4 and 5 follow distinct patterns in the different oils. Component 4 presents characteristics of conjugated *cis-trans* or *trans-trans* double bonds in the IR spectra at 987 and 950 cm^{-1} and a band around 975 cm^{-1} . The latter could derive from some mixing with component 3 due to rotational ambiguity. Component 4 also has IR bands at 1690 and 1640 cm^{-1} characteristic of unsaturated aldehydes. In Raman it has a very strong band at 1654 cm^{-1} (with a shoulder at 1640 cm^{-1}) and a small one at 1160 cm^{-1} . These two bands can be found in the Raman spectrum of fatty acids with conjugated double bond systems, such as the conjugated linoleic isomers. This component increases continuously in corn, sunflower and safflower oil, which are rich in linoleic acid. In rapeseed, peanut and olive oil it reaches a maximum which is followed by a decrease. The concentration of this component in olive oil is always lower than in the other oils. This component reflects the formation of species with conjugated double bonds and α,β unsaturated aldehydes. Component 5 is a minor component in all the oils. It increases at early stages of oxidation in corn, sunflower and safflower oil and then decreases. In rapeseed a monotonous increase of this component is observed, whereas in peanut oil it remains practically constant and increases only at the end of the oxidation process. The most different feature of this component is the band at 1635 cm^{-1} in Raman. It shares similar spectral features with component 4: the bands at 1160 cm^{-1} in Raman and 1690 , 1640 cm^{-1} in IR, which are characteristic of conjugation. The IR spectra of these two components are very similar but in Raman the presence of the 1635 cm^{-1} distinguishes them, giving a different concentration profile. Despite a certain mixing with component 4, component 5 could reflect the formation of species containing conjugated trienes. Few of these species are produced during oxidation, but they can be observed due to their exceptional Raman scattering intensity.

Finally, in the individual analysis of olive oil an additional minor species was detected. Its concentration decreased fast at the beginning of oxidation. Despite both IR and Raman spectra were noisy, characteristic spectral features of highly conjugated compounds like carotenoids were clearly seen: 1620 and 1730 cm^{-1} in IR and especially 1525 and 1155 cm^{-1} in Raman. This component is not detected in the combined analysis because

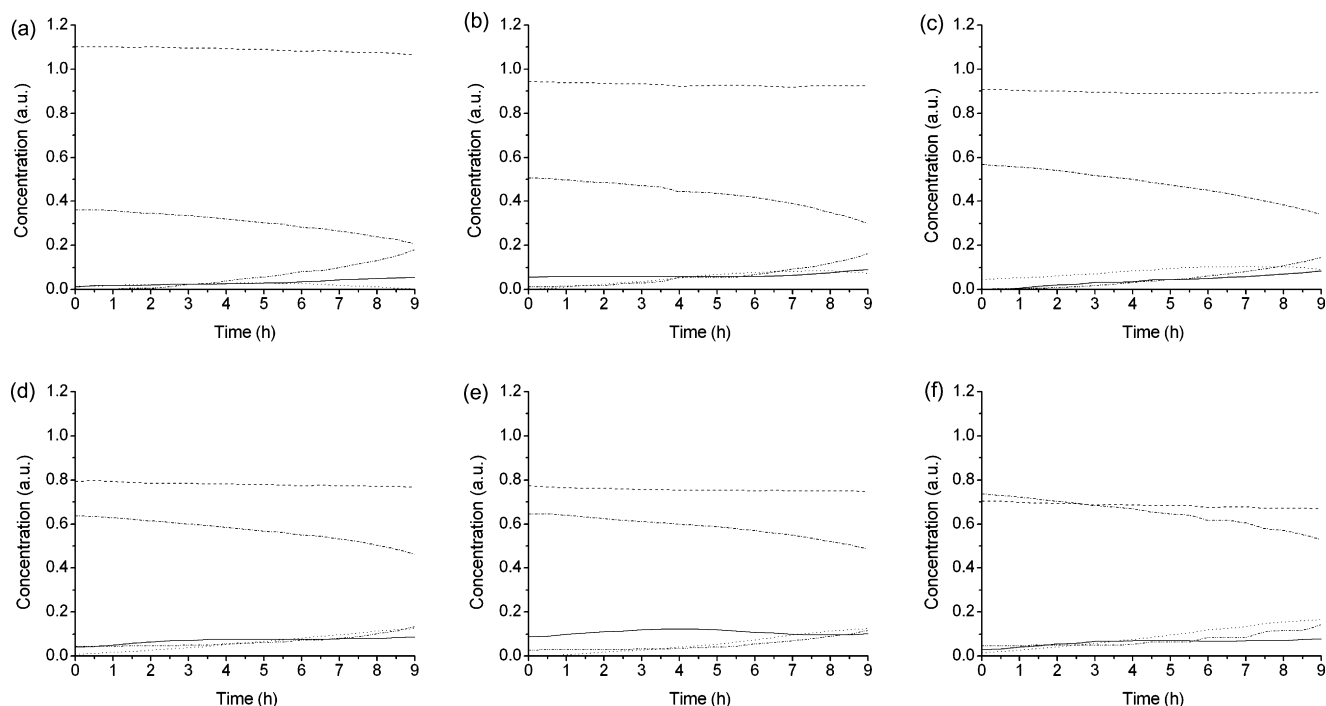


Fig. 8. MCR-ALS results from oil oxidation experiments. Concentration profiles for: (a) olive (b) peanut, (c) rapeseed, (d) corn, (e) sunflower and (f) safflower oil. Component 1 (dash), component 2 (dash dot), component 3 (dash dot dot) component 4 (dot) component 5 (solid).

of its very low contribution to the total variance. Carotenoids are natural antioxidants only present in virgin olive oil and not in refined oils. Furthermore, they are present in very low concentration and can only be detected because they are extremely strong Raman scatterers.

4. Summary and conclusions

With the help of two-dimensional correlation maps, and especially the moving window approach, the information contained in the infrared and Raman spectra about the different events taking place during oxidation of edible oils has been easily visualized. Bands due to the formation of carbonyl compounds, formation of *trans* double bonds and conjugated systems were identified. These results are in agreement with previous studies carried out using spectral subtraction of the non-oxidized oil, but with two-dimensional correlation spectroscopy it was possible to obtain better resolution of the bands. Using MCR-ALS an attempt to model the system was performed. Even considering that rotational and intensity ambiguities exist and the solution found is only one of a number of possible solutions, useful information about the system was obtained. Furthermore, both chemometric approaches lead to similar results concerning: (a) formation of conjugated double bond systems of different type at the beginning of oxidation, (b) formation of saturated carbonylic compounds and isolated *trans* double bonds with a similar kinetic, slowly at the beginning of the oxidation process and continuously during the whole oxidation period, (c) assignments of bands made with two-dimensional correlation spectroscopy were confirmed with the pure spectra of the components obtained with MCR-ALS, and (d) differences in the

behavior of the oils during the oxidation process were observed, and in most of the cases they could be associated with differences in the fatty acid composition.

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