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Study by means of ^{1}H nuclear magnetic resonance of the oxidation process undergone by edible oils of different natures submitted to microwave action

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Abstract

The oxidation processes of virgin olive, corn and linseed oils, repeatedly submitted to the effect of microwave heating until the temperature reached 190 °C, have been studied by means of ^{1}H nuclear magnetic resonance. These oxidation processes are very different to those produced at 70 °C with aeration; differences have been found not only in the rate of degradation and in the oxidation mechanisms but also in the nature and proportions of the aldehydes generated. It has also been shown that these oxidative conditions affect the three oils studied in very different ways; virgin olive oil is the least affected by this process and in addition its degradation produces lower proportions of harmful aldehydes.

Keywords: Virgin olive; Corn and linseed oils; Oxidation process; Microwaves; ^{1}H Nuclear magnetic resonance; Acyl groups; Primary oxidation compounds; Secondary oxidation compounds; Aldehydes

1. Introduction

The most important cause of oil and fat deterioration is the oxidation process which not only reduces both shelf life and the nutritional value of these products but also produces toxic compounds. During this process a great number of changes occur in the sample as consequence of the incorporation of oxygen into the triglyceride structure, and the generation of very reactive species which causes the breakdown of the acyl group chains producing volatile and semi-volatile molecules of different natures, as well as reactions between different acyl groups chains to give oligomeric or polymeric systems. Classical methods of studying this process provide partial information, referring only to the concentration of one class of compounds present in the complex mixture formed during the oxidation process, but none of them give a picture of the total mixture; this fact, combined with the great number of inherent drawbacks of these methods, such as the poor reproducibility of some, or the difficulty in knowing the real significance of the parameters obtained in others (Frankel, 2001), shows the necessity to search for new methods to study these complex processes in depth. With this in mind, the usefulness of Fourier transform infrared spectroscopy as well as of the ^{1}H nuclear magnetic resonance, NMR, has been studied with positive results (Guillén & Cabo, 1999, 2000, 2002; Guillén & Ruiz, 2001, 2004a, 2005).

^{1}H nuclear magnetic resonance has proved to be a very valuable tool which allows one to follow not only the degradation of acyl groups but also, at the same time, the formation and degradation of primary oxidation compounds as well as the formation and evolution of secondary oxidation products throughout the

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oxidation process. This technique has proved the formation of the genotoxic and citotoxic 4-hydroperoxy-, 4-hydroxy- and 4,5-epoxy-*trans*-2-alkenals in the oxidation process undergone by sesame oil and oils rich in linolenic acyl groups at 70 °C with aeration (Guillén & Ruiz, 2004a, 2005). The fact that these harmful compounds, detected in cells and tissues, and known to be responsible for numerous diseases (Eckl, 2003; Esterbauer, Schaur, & Zollner, 1991; Lee, Oe, & Blair, 2002; Lee, Rindgen, Bible, Hajdu, & Blair, 2000; Liu, Raina, Smith, Sayre, & Perry, 2003; Zarkovic, 2003), can be present in foods and can be ingested (Grootveld et al., 1998; Indart et al., 2002; Kanazawa, Kanazawa, & Natake, 1985; Kim, Gallaher, & Csallany, 1999), introduces a new perspective in relation to the safety of both oxidation processes and oils. As a consequence of these results it has been considered of great interest the study of both oxidation processes and oils in function of their potentiality to produce these harmful compounds.

Changes produced in oils by effect of microwave heating have been evaluated by the determination of properties such as density, viscosity, refractive index, or of parameters such as peroxide values, absorptions at 232 and 270 nm, or by changes in the content of squalene, tocoferols, *trans*-isomers or polar compounds (Albi, Lanzon, Guinda, Leon, & Perez-Camino, 1997; Caponio, Pasqualone, & Gomes, 2002; Marinova, Yanishlieva, Toneva, Psomiadou, & Tsimidou, 2001; Rugulska-Ilow, Ilow, & Szymczak, 1998); however, data on oxidative stability of some edible oils submitted to microwave heating are not always in agreement. In addition, to the best of our knowledge, a study of the ability of this degradative process to yield aldehydes from edible oils of very different natures has not been carried out. In this paper, the study of the oxidation process of virgin olive oil as well as of corn and linseed oils when submitted to the effect of microwaves is undertaken with a double purpose: firstly, to compare this oxidation process with that produced at 70 °C with aeration and, in addition, to compare the behaviour of these three oils during the microwave action, not only in relation to oxidation rate but also to the nature of the aldehydes produced. The study has been accomplished by ^{1}H nuclear magnetic resonance.

2. Materials and methods

2.1. Samples and standards

Oils subject of study were virgin olive, corn and linseed oils acquired from local supermarkets. Table 1 gives the proportions of the different acyl groups in these oils determined from ^{1}H nuclear magnetic resonance, as has been described in previous studies (Guillén & Ruiz, 2003a, 2003b). Standard compounds such as: heptanal, octanal, *trans*-2-heptenal, *trans*-2-octenal, *trans*,*trans*-2,4-heptadienal, *trans*,*trans*-2,4-nonadienal and *trans*, *trans*-2,4-decadienal were acquired from Aldrich (Milwaukee, WI, USA); 4-hydroxi-*trans*-2-nonenal was acquired from Merck (Whitehouse Station, NJ, USA), and 4-hydroxy-*trans*-2-hexenal and 4-oxo-*trans*-2-nonenal were acquired from Cayman Chemicals (Ann Arbor, MI, USA).

Table 1
Proportions of the different acyl groups in virgin olive, corn and linseed oils

	Linolenic	Linoleic	Oleic	Saturated
Virgin olive	0.7	6.1	80.3	12.9
Corn	0.8	48.6	35.2	15.4
Linseed	55.6	15.6	16.4	12.4

2.2. Samples oxidation

Ten grams of oil were weighed in crystal Petri-dishes of 80 mm diameter and 15 mm high and placed symmetrically in a microwave oven. The microwave oven operated at maximum frequency of 2450 MHz. The samples were repeatedly microwaved for time periods of 10 min, up to 240 min of total oxidation time. Every 10 min the microwave oven was stopped in order to avoid temperatures higher than 190 °C. The experiments were carried out in duplicate, and the process was periodically monitored by ^{1}H NMR.

2.3. ^{1}H nuclear magnetic resonance

The ^{1}H NMR spectra were recorded on a Varian 300 Plus spectrometer operating at 299,862 MHz. Each oil sample, weighing 0.2 g was mixed with 400 μl of deuterated chloroform and a small proportion of TMS as internal reference; this mixture was introduced into a 5 mm diameter tube. The acquisition parameters were: spectral width 5000 Hz, relaxation delay 3 s, number of scans 32, acquisition time 3.744 s and pulse width 90°, with a total acquisition time of 3.37 min. The experiment was carried out at 25 °C. Spectra were acquired periodically throughout the oxidation process. The assignment of the signals was made as in previous studies (Guillén & Ruiz, 2003c; Johnson & Schoolery, 1962; Miyake, Yokomizo, & Matsuzaki, 1998; Sacchi, Addeo, & Paolillo, 1997) and is given in Table 2. The area of the signals was determined by using the equipment software and the integrations were made three times to obtain average values. All figures of ^{1}H NMR spectra or of expanded ^{1}H NMR spectra regions were plotted at a fixed value of absolute intensity to be valid for comparative purposes.

Table 2
Assignment of the signals of oils ^{1}H NMR spectra

Signal	Chemical shift (ppm)	Functional group
1	0.83–0.93	–CH_3 (saturated, oleic and linoleic acyl groups)
2	0.93–1.03	–CH_3 (linolenic acyl groups)
3	1.22–1.42	–$(CH_2)_n$– (acyl groups)
4	1.52–1.70	–OCO–CH_2–CH_2– (acyl groups)
5	1.94–2.14	–CH_2–CH=CH– (acyl groups)
6	2.24–2.36	–OCO–CH_2– (acyl groups)
7	2.70–2.88	=HC–CH_2–CH= (acyl groups)
8	4.05–4.35	–CH_2OCOR (glyceryl groups)
9	5.20–5.26	>CHOCOR (glyceryl groups)
10	5.26–5.40	–CH=CH– (acyl groups)

The signal number agrees with those in Fig. 1.

3. Results and discussion

The evaluation of the changes suffered by the several oils submitted to degradative conditions was carried out, as stated above by ^{1}H nuclear magnetic resonance. Fig. 1(a)–(c) shows the region between 0 and 5.5 ppm of the ^{1}H NMR spectra of virgin olive, corn and linseed oils, respectively, at different times during the microwave heating. This region contains all the main signals of unoxidized edible oils, which were assigned in the early sixties in the pioneer paper of Johnson and Schoolery (1962) to the different classes of hydrogen atoms, as indicated in Table 2. In Fig. 1(a), corresponding to virgin olive oil, a slight diminution in the intensities of the signals of the allylic and olefinic protons, owing to oleic, linoleic and linolenic groups, (signals 5 and 10, respectively) in relation to that of signal 6 (owing to methylene protons in α position in relation to the carbonyl group) can be observed, with the naked eye, after 240 min of microwave action; this fact indicates that a certain proportion of unsaturated acyl groups of the virgin olive oil has been degraded, although the signal of bis-allylic protons due to linoleic and linolenic groups (signal 7) is visible at the end of the experiment indicating that one or both of these latter acyl groups have not been totally degraded.

Likewise, in Fig. 1(b) a clear lessening in the intensities of allylic, bis-allylic, and olefinic protons (signals 5, 7, and 10, respectively) in relation with that of signal 6 is also observable with the naked eye, after 240 min of microwave heating, showing that unsaturated acyl groups have suffered significant degradation in the process. In the same way, Fig. 1(c) shows a great diminution in the intensities of signals 2, 5, 7, and 10 (due to methylic protons of linolenic groups, and of allylic, bis-allylic and olefinic protons, respectively, owing to oleic, linoleic and linolenic acyl groups) in relation with that of signal 6, indicating that in linseed oil submitted to the microwave heating, for 240 min, a significant proportion of double bonds have been destroyed, especially those of the linolenic groups. To sum up, in none of the three oils, microwaved for 240 min, there has been a total disappearance of any of the 10 signals in Fig. 1.

Much more detailed information can be extracted from the enlargement of some of the above cited signals. Fig. 2(a) gives the enlargement of the ^{1}H NMR spectra signals of methylic hydrogen atoms of triolein plus triestearin, trilinolein and trilinolenin, and Fig. 2(b)–(d) gives the enlargement of this same region of virgin olive, corn and linseed oils ^{1}H NMR spectra, respectively, throughout the oxidation process previously described (signals 1 and 2 in Fig. 1). This region provides information about the proportions of linolenic, linoleic and of

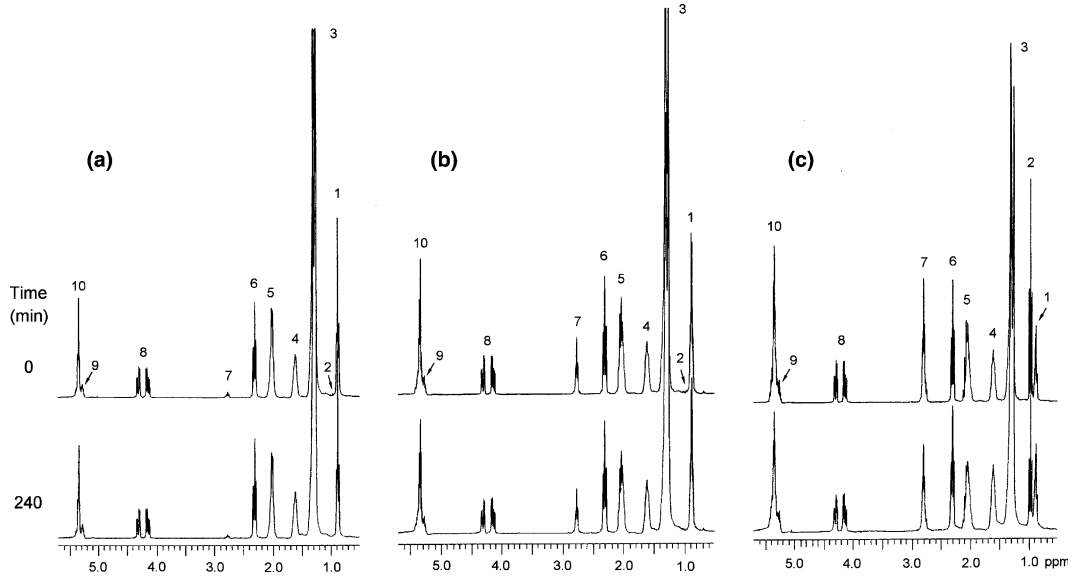

Fig. 1. Region between 0.5 and 5.5 ppm of the ^{1}H NMR spectra of: (a) virgin olive oil, (b) corn oil and (c) linseed oil, throughout the oxidative process.

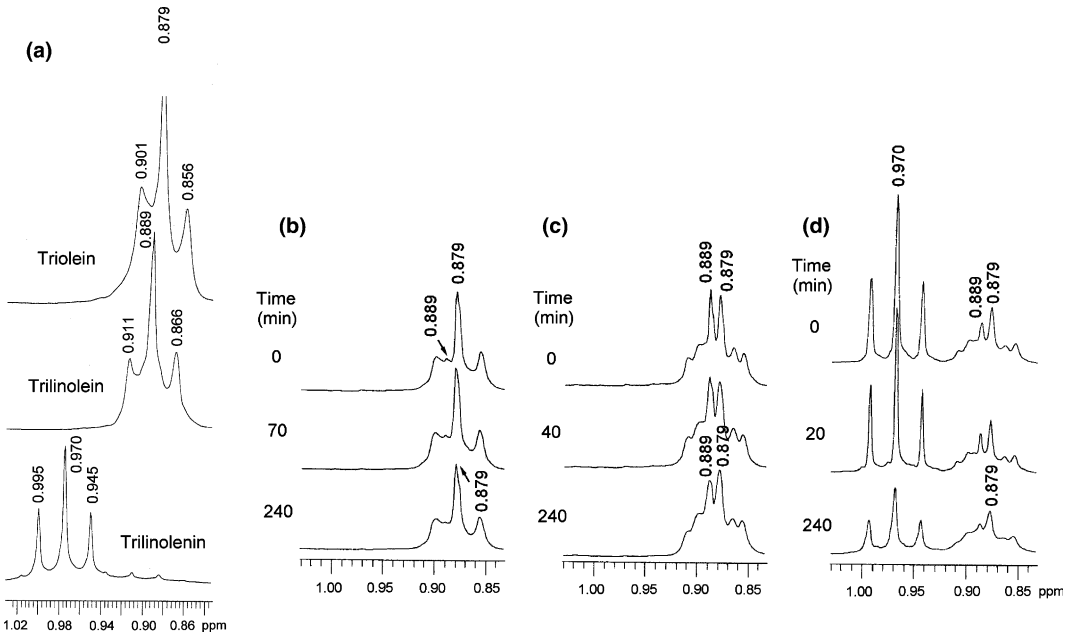

Fig. 2. Expansions of the methylic region, between 0.83 and 1.03 ppm, of the ^{1}H NMR spectra of: (a) triestearin, triolein, trilinolein and trilinolenin, (b) virgin olive oil, (c) corn oil, and (d) linseed oil, at different times under microwave heating.

oleic plus saturated acyl groups in the oils throughout the experiment. Fig. 2(b) shows that this signal, in unoxidized virgin olive oil (time 0), is mainly due to peaks corresponding to oleic plus saturated acyl groups (at 0.856, 0.879 and 0.901 ppm) and it also contains a small peak due to linoleic acyl groups (0.889 ppm), in agreement with data in Table 1; however, after microwave heating, the intensity of the peak owing to linoleic group diminishes in such a way that after 240 min it has almost disappeared, although it is still observable. From this observation, it cannot be inferred that in this process the only degraded acyl group is linoleic.

Fig. 2(c) gives the same region of corn oil throughout the oxidation process; as above this signal shows that undegraded corn oil is made up (time 0) mainly of linoleic (peaks at 0.991, 0.889 and 0.866 ppm) and of oleic plus saturated (peaks at 0.901, 0.879 and 0.856 ppm) acyl groups, the first peaks being higher than the second, in agreement with data in Table 1; however, after 240 min of microwaving, this signal changes in such a way that the intensities of these peaks suffer an inversion, showing that linoleic acyl groups have degraded much more extensively than oleic plus saturated acyl groups. Finally, Fig. 2(d) gives the signal of methylic protons of linseed oil; as can be observed (time 0) this oil contains an important proportion of linolenic acyl groups (peaks at 0.945, 0.970 and 0.995 ppm) and in it the proportion of oleic plus saturated is higher than that of linoleic acyl groups, in agreement with data in Table 1. In Fig. 2(d), it can also be observed that the microwaving process affects especially the linolenic acyl groups, a significant proportion of them being degraded after 240 min, as the reduction of the intensity of their signal shows, and to a lesser extent also affects linoleic groups, whose intensity also suffers a clear reduction. From all the above said it is evident that after 240 min of microwave action, linoleic groups are degraded in all three oils, and linolenic groups are degraded to a great extent in linseed oil.

Fig. 3(a) gives the signals of the allylic protons of oleic (triolein), linoleic (trilinolein) and linolenic (trilinolenin) acyl groups and Fig. 3(b)–(d) shows the evolution of allylic proton signals of the virgin olive, corn and linseed oils (signal 5 in Fig. 1) throughout the oxidation process. This signal is due to the overlapping of signals of oleic (peaks at 2.000 and 2.020 ppm), linoleic (peaks at 2.036 and 2.057 ppm) and linolenic (peaks at 2.073, 2.097 and 2.122 ppm) acyl groups (see Fig. 3(a)).

Fig. 3(b) shows the evolution of this signal in virgin olive oil throughout the degradation process. It can be observed that this signal in the unoxidized virgin olive oil (time 0) is mainly constituted by the peaks of oleic groups (at 2.020 and 2.000 ppm), peaks of linoleic or linolenic groups being scarcely observable; after the microwave action, the intensity of this signal suffers a lessening indicating that a certain proportion of oleic acyl groups has been degraded. Fig. 3(c) shows the allylic proton signal of corn oil. This signal is mainly made up of linoleic (peaks at 2.080, 2.057, 2.036 and 2.016 ppm) and oleic (peaks at 2.000 and 2.020 ppm) groups, the contribution of the first group being more important than that of the second, in agreement with the corn oil composition. At time 0, peaks of linoleic acyl groups show higher intensity than those of oleic

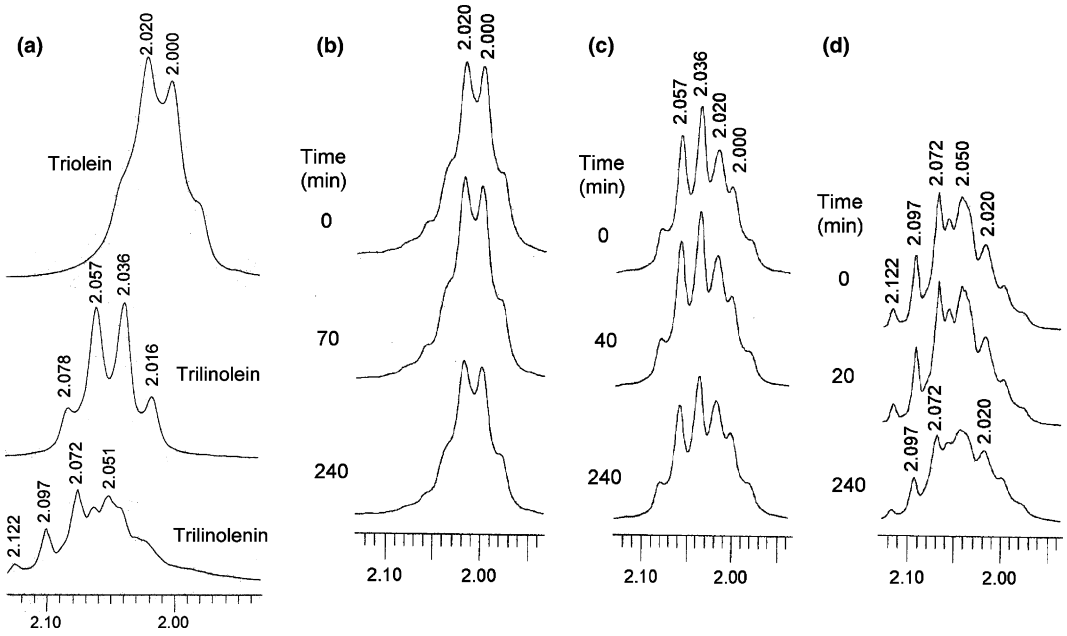

Fig. 3. Expansions of the allylic region, between 1.93 and 2.13 ppm, of the ^{1}H NMR spectra of: (a) triolein, trilinolein and trilinolenin, (b) virgin olive oil, (c) corn oil, and (d) linseed oil, at different times under microwave action.

groups but, after 240 min, this difference in the intensity (or height) of the peaks of both groups diminishes, showing that in corn oil linoleic are degraded more quickly than oleic acyl groups, in agreement with that observed in the methylic region (signal 1). Finally, Fig. 3(d), at time 0, shows the signal of allylic protons in unoxidized linseed oil. From the observation of Fig. 3(d) it is evident that, after 240 min under degradative conditions, the intensities of the allylic protons of linolenic groups (peaks at 2.072, 2.097 and 2.122 ppm) are considerably reduced, oleic groups being much less affected. In summary, from observation of Fig. 3 it can be inferred that in olive oil a certain proportion of oleic acyl group is degraded, whereas linoleic in corn oil and linolenic in linseed oil are the main acyl groups affected.

Fig. 4(a) shows the bis-allylic proton signals of linoleic (trilinolein) and of linolenic (trilinolenin) acyl groups, and Fig. 4(b)–(d) gives the signal of bis-allylic protons in virgin olive, corn and linseed oils at different periods of time throughout the experiment. Bis-allylic proton signals of these oils are formed only of linolenic (peaks at 2.819, 2.799 and 2.781 ppm) and linoleic (peaks at 2.785, 2.765 and 2.745 ppm) acyl groups (see Fig. 4(a)). Fig. 4(b) shows the enlargement of this virgin olive oil signal at different times during the process; it can be observed that the intensities of both groups of peaks diminish after 240 min under degradative conditions, however, at this time, peaks of linoleic groups are still present as well as very small signals of linolenic groups, showing that these groups are not totally degraded. In corn oil this signal is, basically, due to the linoleic group, as Fig. 4(c) shows; the intensity of this signal decreases with time indicating its degradation under these oxidative conditions, in agreement with that observed in the other corn oil signals above mentioned. Finally, Fig. 4(d) shows the enlargement of bis-allylic proton signals of linseed oil spectra throughout this oxidation process. It can be observed that linolenic suffer higher degradation than linoleic acyl groups also in agreement with the results above. In summary, from Fig. 4 it can be inferred that linoleic groups are not totally degraded at the end of the experiment in virgin olive oil, however these acyl group suffers important degradation in corn oil as do linolenic acyl groups in linseed oil, in agreement with what has been observed in the other spectral signals.

While all these changes are produced the appearance of some small signals is observed at 1.55, 2.55, 2.65 and 2.89 ppm in the spectra of oxidized virgin olive oil and some of them in that of corn oil, though they are totally absent in the spectra of linseed oil. Some of these signals are attributable to epoxy protons (Pouchert & Behnke, 1993).

From the integration of the main signal areas above mentioned, the proportions of the several acyl groups can be determined (Guillén & Ruiz, 2003a, 2003b), at different intervals of time, under degradative conditions. The results thus obtained are represented in Fig. 5(a)–(c), which shows the evolution of the different acyl groups, throughout the microwave induced oxidation process, of virgin olive, corn and linseed oils, respectively. It can be observed that in virgin olive oil the highest level of degradation is undergone by the oleic acyl groups, in corn oil by the linoleic and in linseed by the linolenic acyl groups; however none of these acyl groups

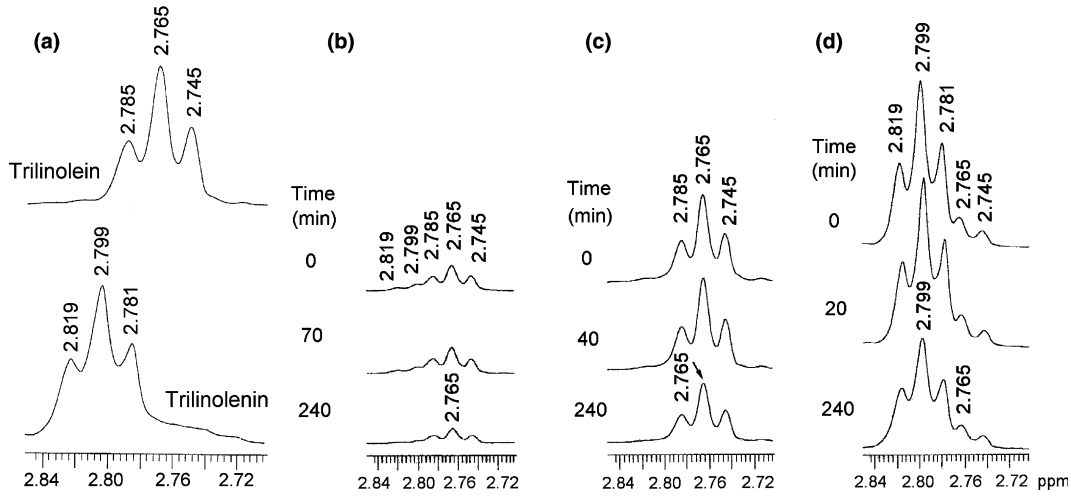

Fig. 4. Expansions of the bis-allylic region, between 2.72 and 2.84 ppm, of the ^{1}H NMR spectra of: (a) trilinolein and trilinolenin, (b) virgin olive oil, (c) corn oil, and (d) linseed oil, at different times during microwave heating.

are totally degraded in any of the three oils (although the linolenic proportion is very small in virgin olive and in corn oils at the end of the experiment). In addition, it can also be observed from these figures that in virgin olive and in corn oils, the proportion of the unsaturated acyl groups, referred to the current content of acyl groups, diminishes throughout the process in a quite similar proportion (near to 10%), and much smaller than that of linseed oil (near 20%).

As the acyl groups undergo degradation new compounds are generated. In oxidation processes of oils of varied composition, provoked at 70 °C with aeration, the degradation of the acyl groups generates primary oxidation products, or hydroperoxides, which give ^{1}H NMR broad signals, between 8.3 and 8.9 ppm, owing to the hydroperoxyde protons, and several multiplet signals, between 5.5 and 6.6 ppm, owing to *cis*,*trans* (multiplets near 6.00 and 6.55 ppm) and to *trans*,*trans* (multiplets near 5.70 and 6.20 ppm) protons of their dienic conjugated systems. The concentration of these primary oxidation products increases as the oxidation advances, reaching a maximum value after which a sharply degradation of these products occurs, generating secondary oxidation products, except in linseed oil oxidation in which signals of both primary and secondary oxidation products appear at the same time (Guillén & Ruiz, 2004a, 2005). Among the generated secondary oxidation products there are aldehydes, which give signals in the ^{1}H NMR spectrum between 9.3 and 9.9 ppm (Claxson et al., 1994; Guillén & Ruiz, 2004a, 2005; Haywood et al., 1995; Sacchi, 2001). If the degradation process caused by microwaving takes place in a similar way to the oxidation process provoked at 70 °C with aeration, the above mentioned primary and secondary oxidation products should also be produced giving signals, in a sequential way, in the same spectral regions above mentioned (Guillén & Ruiz, 2004a, 2005).

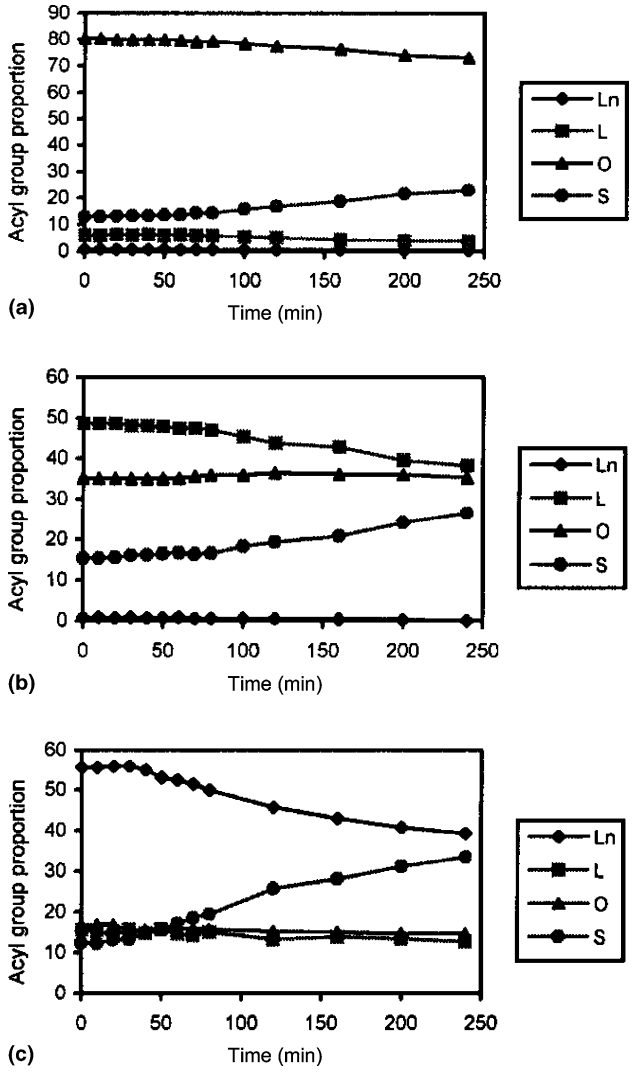

Fig. 5. Evolution of the proportions of the different acyl groups, linolenic (Ln), linoleic (L), oleic (O) and saturated (S), throughout the oxidation process of: (a) virgin olive oil; (b) corn oil; (c) linseed oil.

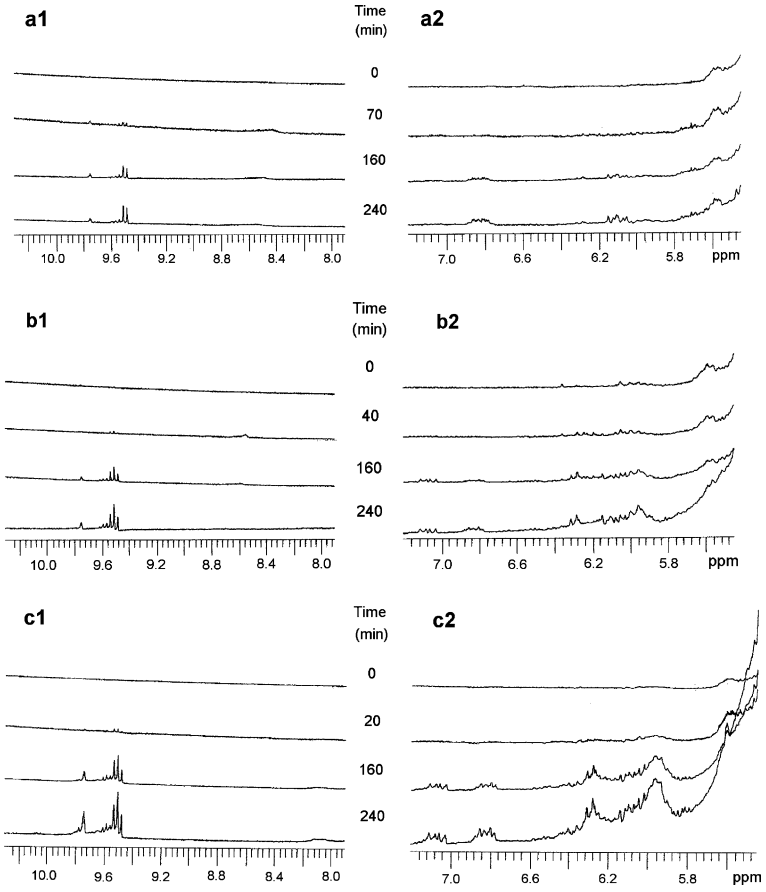

Fig. 6. Expanded regions between 7.9 and 10.3 ppm and between 5.4 and 7.2 ppm of the ^{1}H NMR spectra of: (a1 and a2) virgin olive oil, (b1 and b2) corn oil, and (c1 and c2) linseed oil, at different times during microwave heating.

Fig. 6 shows the spectral regions between 5.4 and 7.2 ppm and between 7.9 and 10.3 ppm of the spectra of virgin olive (Fig. 6(a1) and (a2)), corn (Fig. 6(b1) and (b2)), and linseed (Fig. 6(c1) and (c2)) oils at different times during the process. It can be observed that this process of degradation occurs in a very different way to that at 70 °C with aeration. The first significant fact is that only very small and rare hydroperoxide signals appear, between 8.4 and 8.6 ppm, in virgin olive oil at minute 70, and in corn oil at minute 40, which are not accompanied by signals of protons of their dienic conjugated systems; simultaneously with the appearance of these incipient signals of hydroperoxides, signals of aldehydes, between 9.4 and 9.8 ppm, appear in both oils. In linseed oil spectra, tentative signals of hydroperoxides, between 8.0 and 8.2 ppm, appear simultaneously with or somewhat later, than the aldehydes signals, which appear at 20 min, between 9.4 and 9.8 ppm.

The absence of significant concentrations of hydroperoxides in the oils submitted to the microwave heating together with the absence of dienic systems in this oil degradation process proves that this process evolves in a very different way to that produced at 70 °C with aeration. In addition to these differences, it is also worth noting the rapid generation of aldehydes in the oils submitted to microwaving. All these facts could be due to, in this process, the degradation rate of hydroperoxides being similar to their rate of formation, for which reason they are almost absent in the oil matrix during the process.

Table 3 gives the chemical shifts of the signals of the compounds generated in this degradation process from the three mentioned oils together with their assignment, multiplicity and their appearance time. It can be observed that the rate of generation of aldehydes is higher in linseed oil than in corn oil, and higher in corn oil than in olive oil, showing that this last oil has the highest oxidative stability of the three oils studied.

Aldehydes, in addition to signals of the aldehydic proton, give signals of olefinic protons: thus, virgin olive oil gives multiplet signals near 6.10 and 6.85 ppm, owing to olefinic protons of *trans*-2-alkenals (Fig. 6(a2)), showing that these are the main aldehydes generated from the virgin olive degradation process during heating by microwave action; and in addition to these signals, corn and linseed oils spectra (Fig. 6(b2) and (c2)) also have multiplet signals near to 6.08, 6.30 and 7.10 ppm owing to olefinic protons of *trans*,*trans*-2,4-alkadienals

Table 3
Chemical shift assignment of the ^{1}H NMR signals of the products generated in the oxidation process of virgin olive, corn and linseed oils under microwave action, together with their multiplicities and intervals of existence in the process

Compounds	Chemical shift (ppm)	Functional group	Signal appearance time		
			Virgin olive	Corn	Linseed
Hydroperoxides	8.4–8.6 (bs)	–OO*H* (hydroperoxide group)	70	40	–
Hydroperoxides derivatives	8.0–8.2 (bs)	–OO*H* (hydroperoxide group)	–	–	120
trans-2-alkenals	9.480 and 9.506 (d)	–C*H*O (aldehydic group)	70	40	20
trans,*trans*-2,4-alkadienals	9.507 and 9.533 (d)	–C*H*O (aldehydic group)	70	40	20
cis, trans-2,4-alkadienals	9.580 and 9.606	–C*H*O (aldehydic group)	–	100	20
n-alkanals	9.748 (t)	–C*H*O (aldehydic group)	70	40	20
4-hydroxy-*trans*-2-alkenals	9.560 and 9.586 (d)	–C*H*O (aldehydic group)	120	80	50
4,5-epoxy-*trans*-2-alkenals	9.538 and 9.564 (d)	–C*H*O (aldehydic group)	100	80	40
Short chain alkanals or oxo-alkanals	9.778 (t)	–C*H*O (aldehydic group)	–	200	80

bs: broad signal; d: doublet; t: triplet; m: mutiplet.

showing that these diunsaturated aldehydes are formed in significant proportions in the degradation of these oils and especially in that of linseed oil. Furthermore, in corn and linseed oil spectra, an unassigned multiplet signal, at 5.95 ppm, appears in very advanced stages of the process, which overlaps with signals of *trans*-2-alkenals at 6.10 ppm and of *trans*,*trans*-2,4-alkadienals at 6.08 ppm.

Another very important difference between the degradation process caused by the microwave heating and that produced at 70 °C with aeration, concerns the nature and proportions of the aldehydes generated. The ^{1}H NMR signals of the aldehydes generated during the microwave heating of virgin olive, corn and linseed oils and their evolution throughout the process are shown in Fig. 7. It can be observed that among the generated aldehydes there are *trans*-2-alkenals exhibiting the doublet (**a**) at 9.480 and 9.506 ppm and *trans*,*trans*-2,4-alkadienals giving a doublet signal (**b**) at 9.507 and 9.533 ppm, which partially overlaps with the doublet of *trans*-2-alkenals. The assignment of these signals to *trans*-2-alkenals and to *trans*,*trans*-2,4-alkadienals has been made by using *trans*-2-heptenal, *trans*-2-octenal, *trans*,*trans*-2,4-heptadienal, *trans*,*trans*-2,4-nonadienal and *trans*,*trans*-2,4-decadienal as standard compounds. Furthermore, 4,5-epoxy-*trans*-2-alkenals are also formed in this process and exhibit a doublet signal (**c**) at 9.538 and 9.564 ppm which overlaps partially with the doublet (**b**) of *trans*,*trans*-2,4-alkadienals. The assignment of the signal of 4,5-epoxy-*trans*-2-alkenals was tentatively made in agreement with spectral data provided by I.A. Blair and J. Arora and with data published by Lin, Fay, Welti, and Blank (1999). In addition, other oxygenated aldehydes, such as 4-hydroxy-*trans*-2-alkenals are also formed; these give a doublet signal (**e**) at 9.560 and 9.586 ppm, which overlaps partially with the doublet (**d**) of 4,5-epoxy-*trans*-2-alkenals; the assignment of the signals of 4-hydroxy-*trans*-2-alkenals has been made by using 4-hydroxy-*trans*-2-nonenal and 4-hydroxy-*trans*-2-hexenal as standard compounds. Finally, a triplet signal (**f**) centred at 9.748 ppm owing

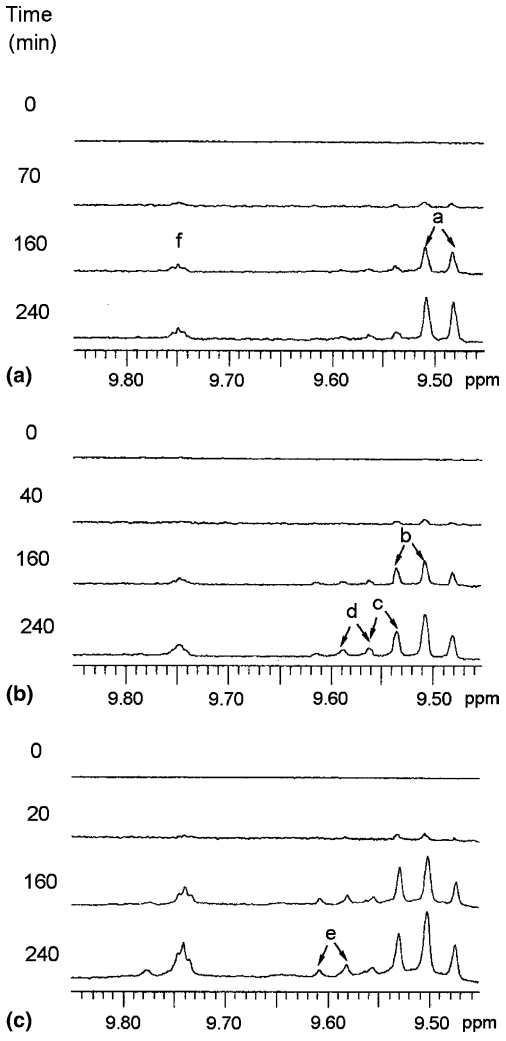

Fig. 7. Enlargement of the region between 9.4 and 10.0 ppm of the ^{1}H NMR spectra at different times under microwave action of: (a) virgin olive oil; (b) corn oil; (c) linseed oil. **a**: doublet signal of *trans*-2-alkenals; **b**: signal of *trans*,*trans*-2,4-alkadienals; **c**: signal attributable to 4,5-epoxy-*trans*-2-alkenals; **d**: doublet signal of 4-hydroxi-*trans*-2-alkenals; **e**: doublet signal attributable to *cis*,*trans*-2,4-alkadienals; **f**: triplet signal of *n*-alkanals, and **g**: triplet signal assignable to *n*-alkanals of small number of carbon atoms, or to oxo-alkanals.

to *n*-alkanals, confirmed by using the standards heptanal and octanal, also appears. In addition, in oils with a significant level of polyunsaturated acyl groups such as corn and linseed oils, a doublet signal (**e**) at 9.585 and 9.611 ppm, which partially overlaps with the doublet of 4-hydroxy-*trans*-2-alkenals, has been tentatively assigned to *cis*,*trans*-2,4-alkadienals in agreement with data provided by other authors (Claxson et al., 1994; Haywood et al., 1995). Finally, in oils with a significant proportion of polyunsaturated acyl groups another triplet signal (**g**) at 9.778 ppm appears, which could be associated either to saturated aldehydes of a small number of carbon atoms or to the aldehydic proton of oxo-alkanals in agreement with different data of the literature (Takeoka, Buttery, & Perrino, 1995). Virgin olive oil does not generate aldehydes corresponding to the doublet signal (**e**) or the triplet signal (**g**).

It should be noticed that the difference in composition of the three oils has as consequence differences in the proportions of the aldehydes generated. Thus, it can be observed that among the three oils, virgin olive oil produces the smallest amount of *trans*,*trans*-2,4-alkadienals and of 4-hydroxy-*trans*-2-alkenals, being *trans*-2-alkenals and alkanals the main aldehydes generated from this oil. From Figs. 6 and 7, it is evident that, as the proportion of polyunsaturated acyl groups in the oil is higher, so is the proportion of *trans*,*trans*-2,4-alkadienals and 4-hydroxy-*trans*-2-alkenals, and that of those aldehydes corresponding to the doublet (**e**) and the triplet (**g**), in such a way that *trans*,*trans*-2,4-alkadienals are produced in higher concentrations than *trans*-2-alkenals in the degradation of corn and linseed oils. In short: alkanals and *trans*-2-alkenals are the main aldehydes generated from virgin olive oil; *trans*, *trans*-2,4-alkadienals, *trans*-2-alkenals and alkanals are the main aldehydes generated from corn and linseed oils; in addition, virgin olive oil is the one that produces the smallest amounts of 4-hydroxy-*trans*-2-alkenals of the three studied oils. These facts are very important because of the different reactivity of the several classes of aldehydes.

It should be also noticed that the degradation process during microwave heating without exceeding 190 °C produces a very different distribution of aldehydes to that produced at 70 °C with aeration; in this latter process the formation of oxygenated aldehydes such as 4-hydroperoxy- and 4-hydroxy-*trans*-2-alkenals was more favoured than the formation of *trans*,*trans*-2,4-alkadienals, whose formation, on the contrary, is favoured in the first process. It is also noteworthy that microwave heating does not produce 4-hydroperoxy-*trans*-2-alkenals, and the pattern of the generated aldehydes and their proportions are maintained constant throughout the process (240 min), although their concentrations increase with time. The absence of 4-hydroperoxy-*trans*-2-alkenals among the aldehydes generated is also consistent with the scarce presence of hydroperoxides in this process; perhaps due to the hidroperoxides being very unstable under these conditions. Furthermore, the fact that the distribution of the aldehydes generated is totally different to that produced at 70 °C with aeration, proves that the degradation mechanisms are different in each process.

In summary not only the rate of generation of aldehydes is different in the three oils but also the class of aldehydes generated. Thus, the formation of 4-hydroxy-*trans*-2-alkenals and 4,5-epoxy-*trans*-2-alkenals, together with the great amount of *trans*,*trans*-2,4-alkadienals generated when oils rich in polyunsaturated acyl groups are submitted to microwaving should be taken into account, because all these aldehydes have been considered cyto- and/or geno-toxic (Blair, 2001; Cabré, Girona, Vallvé, Heras, & Masana, 2003; Lee et al., 2002; Lee et al., 2000; O'Brien, Kaul, McGirr, Drolet, & Silva, 1989) and have been related to several diseases (Esterbauer et al., 1991; Zarkovic, 2003). It is noteworthy that virgin olive oil not only shows the lowest rate of aldehyde generation but also produces the smallest amount of 4-hydroxy-*trans*-2-alkenals and of *trans*,*trans*-2,4-alcadienals of the three oils studied. Finally, it has also been proved that the kind of aldehydes produced by microwave heating is very different from those produced when oils are submitted to lower temperatures with aeration, which should be taken into account in industrial and culinary practices.

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