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Oxidation of corn oil at room temperature: Primary and secondary oxidation products and determination of their concentration in the oil liquid matrix from ^{1}H nuclear magnetic resonance data

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ABSTRACT

The oil liquid matrix of several corn oil samples that have been stored at room temperature in closed receptacles for different periods of time is studied by means of ^{1}H nuclear magnetic resonance (^{1}H NMR), in order to further knowledge about this type of edible oil oxidation. As expected, the degradation of linoleic acyl groups predominates. In samples at early oxidation stages the presence of hydroperoxides and of (*Z,E*) conjugated-dienic systems is demonstrated, the concentration of the first group being higher than that of the second. In addition to these compounds, the presence of hydroxy derivatives supporting (*Z,E*) conjugated-dienic systems, as well as of hydroperoxy derivatives supporting (*E,E*) conjugated-dienic systems, in samples at intermediate and advanced oxidation stages, is also shown. Corn oil samples at advanced stages of oxidation also contain aldehydes, among which there are alkanals, (*E*)-2-alkenals, (*E,E*)-2,4-alkadienals, 4-hydroxy-(*E*)-2-alkenals, 4-hydroperoxy-(*E*)-2-alkenals and 4,5-epoxy-(*E*)-2-alkenals. The concentrations of the different kinds of intermediate compounds above mentioned as well as of the different kinds of aldehydes present in the oil liquid matrix were determined. These latter compounds can be either free or joined to truncated structures of triglycerides. In addition, a principal component analysis between storage conditions and oxidation level of the samples was carried out.

1. Introduction

The most important cause of oil and fat deterioration is oxidation, which not only reduces both shelf life and nutritional value, but also produces toxic compounds. In this process the triglyceride structure undergoes changes, which have as a consequence the formation of a great number of compounds. In general, it has been postulated that first of all primary oxidation compounds, such as hydroperoxides, are generated. Their level is classically evaluated by peroxide value or by their absorbance at 233 nm and 273 nm in the ultraviolet spectrum, although it has been indicated that these parameters are only valid during the first stages of oxidation. It is also accepted that primary oxidation compounds in turn degrade to give so-called secondary oxidation compounds. The concentration of these latter is classically determined by the anisidine value or by the thiobarbituric acid test (TBA), among other methods, although there are doubts about the specific kind of compounds really determined by these tests (Frankel, 2005).

As far as primary oxidation compounds are concerned, it has been shown that ^{1}H Nuclear Magnetic Resonance (^{1}H NMR) can also be used to evaluate the presence in edible oils of hydrogen atoms of hydroperoxy groups, as well as of hydrogen atoms supported on conjugated-dienic systems (Claxson et al., 1994; Guillén & Ruiz, 2004; 2005a, 2005b, 2005c; Guillén & Goicoechea, 2007; Haywood et al., 1995). In addition, it is possible to distinguish within these between (*Z,E*) or (*E,E*) isomerism. Furthermore, it has been proved that hydroperoxide groups and conjugated-dienic systems can be present not only in primary oxidation compounds but also in secondary oxidation products; likewise, it has been shown that among the secondary oxidation compounds, not only alkanals, (*E*)-2-alkenals and (*E,E*)-2,4-alkadienals, but also γ-oxygenated α,β-unsaturated aldehydes can be found (Guillén & Ruiz, 2004, 2005a, 2005b, 2005c; Guillén and Goicoechea, 2007). The genotoxicity and cytotoxicity of these latter compounds is well known (Esterbauer, Schaur, & Zollner, 1991; Guillén & Goicoechea, 2008; Zarkovic, 2003). It is also known that both the evolution of the oxidation of edible oils and fats and the nature and proportions of primary and secondary oxidation products formed in this process depend not only on the oil and fat nature but also on the conditions under which the oxidation takes place.

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Oxidation processes provoked in vegetable oils rich in linoleic acyl groups at 70 °C with aeration have been shown, by means of ^{1}H NMR, to involve the formation of primary oxidation compounds, such as hydroperoxides and (*Z,E*) and (*E,E*) conjugated-dienic systems (Guillén & Ruiz, 2004, 2005a, 2005b, 2005c). These intermediate compounds evolve to give secondary oxidation compounds, resulting from the fragmentation of triglyceride chains, which give rise to compounds of low or high molecular weight. A certain proportion of secondary oxidation products of low molecular weight can remain dispersed in the oil liquid matrix, modifying its original properties, and others can escape towards the surrounding oil atmosphere, producing the typical rancid odour. However, all secondary oxidation compounds of high molecular weight, whether monomers, dimers, oligomers or polymers, remain in the oil liquid matrix, modifying its properties. Previous studies of corn oil oxidation provoked at 70 °C with aeration have also shown that, among the aldehydes formed, alkanals, (*E*)-2-alkenals, 4-hydroperoxy-(*E*)-2-alkenals and 4-hydroxy-(*E*)-2-alkenals, predominate in the oil liquid matrix, whereas (*E,E*)-2,4-alkadienals and 4,5-epoxy-2(*E*)-alkenals are present in much smaller proportions than the others (Guillén & Ruiz, 2005b).

At lower temperatures, not only can the evolution of the oxidation of edible oils and the nature and proportion of the compounds formed be different from that at higher temperatures, but also the rate of the process decreases. Perhaps for this reason there are very few studies concerning oil oxidation at room temperature (Martin-Polvillo, Marquez-Ruiz, & Dobarganes, 2004; Guillén & Goicoechea, 2007; Yang, Chu, & Liu, 2005). In previous papers we have studied, by means of ^{1}H NMR, the composition of certain samples of sunflower oils having different oxidation degrees reached during storage at room temperature in closed containers (Guillén & Goicoechea, 2007). This paper focuses on the study by the same technique of eleven corn oil samples with different oxidation levels, also reached at room temperature in closed containers in the presence of limited amounts of air. The aim of this study is to analyze the information that this technique provides concerning the composition of these corn oil samples in relation to the nature of the intermediate and of some of the secondary oxidation compounds generated in this degradation process; in addition, the determination not only of the proportions but also of the concentrations of all these compounds will be carried out using a new approach, developed for this purpose, based on ^{1}H NMR data. Furthermore, differences and similarities with sunflower oils maintained under similar storage conditions (Guillén & Goicoechea, 2007), as well as with primary and secondary oxidation compounds formed in the oxidation of corn oil at higher temperatures (Guillén & Ruiz, 2005b) will be studied. Finally, principal component analysis will be carried out, comparing storage conditions on the one hand and the proportions of hydroperoxides and aldehydes on the other, in order to study the relationships between them.

2. Materials and methods

2.1. Samples, oxidation conditions and standards

The study was carried out on eleven corn oil samples, acquired from local supermarkets over a period of ten years. The compositions of these oils, when they were acquired, followed the legal requirements of the European Union for edible corn oils. Their molar acyl group proportions ranged between 13% and 14% of saturated acyl groups, between 33% and 34% of oleic acyl groups, between 52% and 53% of linoleic acyl groups and between 0.5% and 1% of linolenic acyl groups. They were stored at room temperature in closed receptacles for different periods of time, under different air–oil volume ratios (AOVR), and different air–oil contact surfaces (CS). The containers were stored inside a cupboard and the room temperature was between 20 °C and 25 °C. The samples consisted of corn oils of the same brand and batch stored with different air–oil volume ratios and/or air–oil contact surfaces, oils of the same brand but from a different batch acquired one or more years later, and oils of different brands.

Under the above storage conditions the corn oil samples underwent an oxidation process. Table 1 gives the storage conditions to which the samples were submitted, such as storage time (ST), air–oil contact surface (CS), air volume (AV), oil volume (OV) and air–oil volume ratio (AOVR). They are arranged in successively higher oxidation stages (from C1 to C11).

Compounds, such as 4-hydroxy-(*E*)-2-nonenal, 4-hydroperoxy-(*E*)-2-nonenal, 4,5-epoxy-(*E*)-2-decenal, 4-hydroxy-(*E*)-2-hexenal acquired from Cayman Chemical (Ann Arbor, MI, USA), and heptanal, octanal, (*E*)-2-heptenal, (*E*)-2-octenal, (*E,E*)-2,4-heptadienal, (*E,E*)-2,4-nonadienal and (*E,E*)-2,4-decadienal, acquired from Sigma–Aldrich (St. Louis, MI, USA), were used as standard compounds for identification purposes.

2.2. ^{1}H nuclear magnetic resonance spectra acquisition and derived data

The ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz. Each oil sample, weighing 0.2 g, was mixed with 400 μl of deuterated chloroform (purity 99.8%) and a small proportion of tetramethylsilane (TMS) as an 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 64, acquisition time 3.744 s and pulse width 90°, with a total acquisition time of 12 min 54 s. The experiment was carried out at 25 °C. The assignment of the signals was made as in previous studies (Guillén & Ruiz, 2004) and is given in Tables 2 and 3. All figures of ^{1}H NMR spectra or of expanded regions were plotted at a fixed value of absolute intensity to be valid for comparative purposes. The area of the new proton signals that appeared as a consequence of the oxidation was determined assigning a value of unity to the area of the non-deuterated chloroform protons signal (7.29 ppm), which has the same concentration in all ^{1}H NMR experiments, as in previous studies (Guillén & Goicoechea, 2007). From these areas the relative molar proportions (rmp) of some functional groups or of groups of compounds, such as hydroperoxides, (*Z,E*) and (*E,E*) conjugated-dienic systems in chains having hydroperoxy groups, (*Z,E*) conjugated-dienic systems in chains having hydroxy groups, alkanals, (*E*)-2-alkenals, 4-hydroperoxy-(*E*)-2-alkenals, 4-hydroxy-(*E*)-2-alkenals, (*E,E*)-2,4-alkadienals and 4,5-epoxy-2(*E*)-alkenals, can be determined; however, this determination is not possible either when the assignment of the signal is unknown or when the signals overlap. Relative molar proportions (rpm), determined in this way,

Table 1
Some of the oxidation conditions during storage of the corn oil samples, such as storage time (ST), air–oil contact surface (CS), air volume (AV), oil volume (OV) and air–oil volume ratio (AOVR).

Sample	ST (months)	CS (cm^2)	AV (cm^3)	OV (cm^3)	AOVR
C1	12	42.2	34.5	951.2	0.036
C2	72	42.2	139.5	845.0	0.165
C3	49	28.3	20.4	983.4	0.021
C4	18	42.2	667.6	316.9	2.107
C5	99	42.2	160.6	823.9	0.195
C6	121	38.5	372.8	500.3	0.745
C7	121	42.2	350.7	633.7	0.553
C8	121	12.6	18.8	62.8	0.300
C9	121	12.6	46.5	35.2	1.321
C10	103	12.6	70.4	11.3	6.230
C11	103	12.6	71.6	10.1	7.090

Table 2
Assignment of the signals of the ^{1}H NMR spectra of the corn oil samples. The signal letters agree with those in Figs. 1 and 2.

Signal	Chemical shift (ppm)	Functional group
A	0.83–0.93	–C**H**$_3$ (saturated, oleic and linoleic acyl group)
B	0.93–1.03	–C**H**$_3$ (linolenic acyl group)
C	1.22–1.42	–(C**H**$_2$)$_n$– (acyl group)
D	1.52–1.70	–OCO–CH$_2$–C**H**$_2$– (acyl group)
E	1.94–2.14	–C**H**$_2$–CH=CH– (acyl groups)
F	2.23–2.36	–OCO–C**H**$_2$– (acyl group)
G	2.70–2.84	=HC–C**H**$_2$–CH= (acyl groups)
H	4.10–4.32	–C**H**$_2$OCOR (glyceryl group)
I	5.20–5.26	>C**H**OCOR (glyceryl group)
J	5.26–5.40	–C**H**=C**H**– (acyl group)

are given in Tables 4 and 6. Likewise, the molar concentrations of these functional groups or groups of compounds were determined in the samples in which they were present, taking the non-deuterated chloroform present in the used deuterated chloroform (0.02%) as standard compound; these molar concentrations are also given in mmol/kg in Tables 4 and 6. These determinations are possible because the area of the ^{1}H NMR signal is proportional to the number of protons which generates the corresponding signal. Each sample was analyzed in triplicate and data shown are average values.

2.3. Statistical study

A principal component analysis of the five variables associated with oxidation conditions (OC), included in Table 1, such as storage time (ST), air–oil contact surface (CS), air volume (AV), oil volume (OV), and air–oil volume ratio (AOVR) was performed. Likewise, principal component analysis of two of the variables associated with the oxidation level (OL) reached by the oil samples determined from ^{1}H NMR spectral data, such as relative molar proportions of total hydroperoxides (HY) and of total aldehydes (AL) given in Tables 4 and 6, was also carried out. Multiple linear regression between the principal component of the variables associated to oxidation level (OL) and the two principal components of the variables associated with oxidation conditions (OC1 and OC2) was also performed. These studies were carried out using the statistical package SPSS (SPSS Inc., Chicago, IL, 2004).

3. Results and discussion

Although corn oil, like sunflower oil, is rich in linoleic acyl groups, it usually contains a slightly or significantly higher proportion of saturated and oleic acyl groups and a smaller proportion of linoleic acyl groups than sunflower oil. In addition, corn oil contains a small proportion of linolenic acyl groups (usually lower than 1%), which are absent in sunflower oil. The typical ^{1}H NMR spectrum of this oil is given in Fig. 1 (sample C1) and it contains ten signals, indicated with different letters, whose assignment is given in Table 2, in agreement with previous studies (Guillén & Ruiz, 2003; Johnson & Shoolery, 1962; Miyake, Yokomizo, & Matsuzaki, 1998; Sacchi, Addeo, & Paolillo, 1997). The enlargement of some of these signals permits one to observe some features of the composition of this oil. Signal A is due to the overlapping of the triplet signals of methylic protons of linoleic, centred at

Table 3
Chemical shift assignment of the ^{1}H NMR signals of some of the products generated in the oxidation process of corn oils stored at room temperature, together with their multiplicities.

Compounds and functional groups	Chemical shift (ppm)	Functional group
Hydroperoxy groups	8.3–8.9 (bs)	OO**H**– (hydroperoxide group)
Unidentified signal 1	7.50 (bs)	??
Unidentified signal 2	8.10 (bs)	??
Hydroperoxy-(*Z*,*E*)-conjugated-dienic systems	6.56, 6.00, 5.58 and 5.45 (m)	–C**H**=C**H**–C**H**=C**H**– (*Z*,*E*) conjugated double bond groups
Hydroxy-(*Z*,*E*)-conjugated-dienic systems	6.48, 5.98, ?? and ?? (m)	–C**H**=C**H**–C**H**=C**H**– (*Z*,*E*) conjugated double bond groups
Hydroperoxy-(*E*,*E*)-conjugated-dienic systems	6.25 and 5.75 (m)	–C**H**=C**H**–C**H**=C**H**– (*E*,*E*) conjugated double bond groups
(*E*)-2-alkenals	9.491 (d) 6.85 (tt) 6.11 (dd)	C**H**O– (aldehydic group) CHO–CH=C**H**– (acyl group) CHO–C**H**=CH– (acyl group)
(*E*,*E*)-2,4-alkadienals	9.527 (d) 7.09 (m) 6.30 (m) 6.08 (dd)	C**H**O– (aldehydic group) CHO–CH=C**H**– (acyl group) CHO–CH=CH–C**H**=C**H**– (acyl group) CHO–C**H**=CH– (acyl group)
Alkanals	9.748 (t)	C**H**O– (aldehydic group)
4-Hydroxy-(*E*)-2-Alkenals	9.577 (d) 6.84 (dd) 6.33 (m)	C**H**O– (aldehydic group) CHO-CH=C**H**– (acyl group)CHO–C**H**=CH– (acyl group)
4-Hydroperoxy-(*E*)-2-alkenals	9.582 (d) 6.80 (dd) 6.33 (m) 8.20 (d)	C**H**O– (aldehydic group) CHO–CH=C**H**– (acyl group) CHO–C**H**=CH– (acyl group) OO**H**– (hydroperoxide group)
4,5-Epoxy-(*E*)-2-alkenals	9.536 (d) 6.56 (dd) 6.40 (dd)	C**H**O– (aldehydic group) CHO–CH=C**H**– (acyl group) CHO–C**H**=CH– (acyl group)

Abbreviations: bs, broad signal, m, multiplet, d, doublet, t, triplet.

Table 4
Relative molar proportions (rmp) and concentrations (mmol/kg) in corn oil of different functional groups corresponding to intermediate oxidation compounds present in corn oil samples oxidized at room temperature in closed containers.

Groups		Samples										
		C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**
Hydroperoxy groups	rmp	–	0.29	0.21	0.30	0.26	0.32	0.42	1.73	2.35	2.63	1.07
	mmol/kg	–	15.68	11.31	16.50	14.42	17.70	22.78	94.61	128.26	143.50	58.67
Hydroperoxy-(*Z*,*E*)-conjugated-dienic systems	rmp	–	0.05	0.06	0.19	0.14	0.23	0.33	0.80	1.16	1.35	n.d.
	mmol/kg	–	2.95	3.94	10.32	7.59	12.51	18.14	43.70	63.37	73.69	n.d.
Hydroxy-(*Z*,*E*)-conjugated-dienic systems	rmp	–	–	–	–	0.03	0.09	0.09	0.60	0.96	1.02	n.d.
	mmol/kg	–	–	–	–	1.64	4.70	5.03	32.83	52.17	55.99	n.d.
Hydroperoxy-(*E*,*E*)-conjugated-dienic systems	rmp	–	–	–	–	–	–	–	0.27	0.60	0.82	n.d.
	mmol/kg	–	–	–	–	–	–	–	14.58	32.83	44.93	n.d.

n.d. = Not determined due to the overlapping of signals.

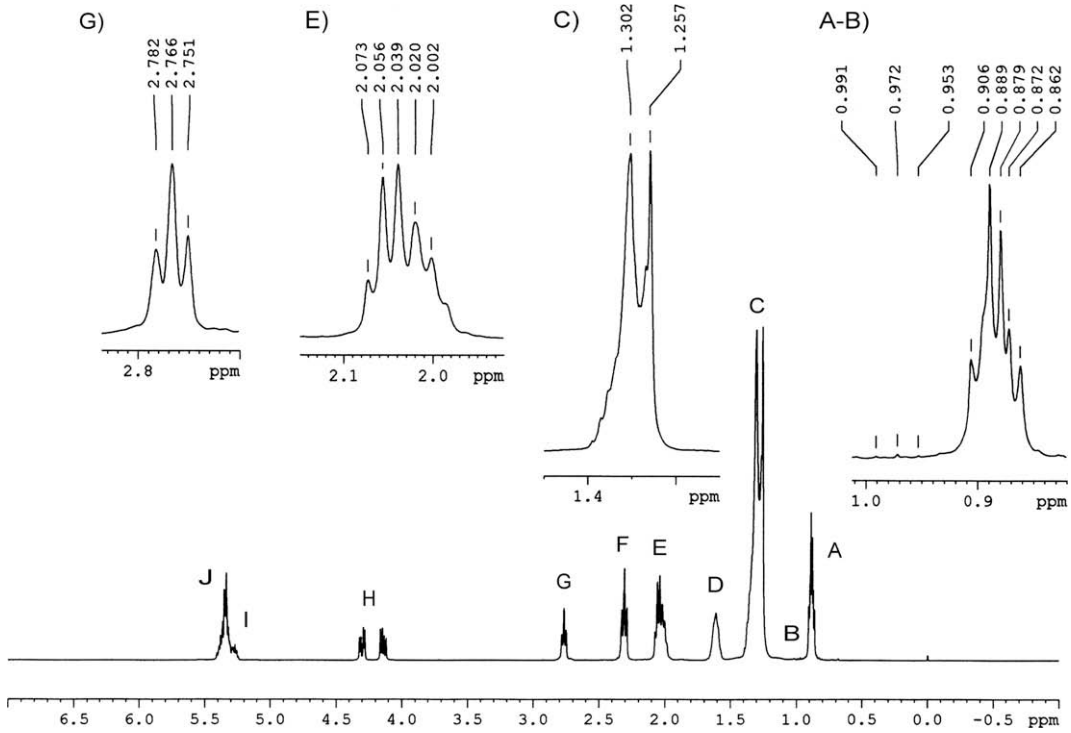

Fig. 1. Typical ^{1}H NMR spectrum of a non-oxidized corn oil (sample **C1**). Signals A, B, C, E and G have been suitably enlarged.

0.889 ppm, and of oleic plus saturated acyl groups, triplet centred at 0.879 ppm (see Fig. 1). This allows one to infer the higher proportion of linoleic than of oleic plus saturated acyl groups, as a result of the higher intensity of the peak at 0.889 ppm than that of the peak at 0.879 ppm, in agreement with previous results (Guillén & Ruiz, 2003). Signal B is the triplet of methylic protons of linolenic acyl groups, centred at 0.972 ppm (Guillén & Ruiz, 2003).

In addition, the proportions of linoleic, oleic and saturated acyl groups are also reflected in the peaks of the signal C due to the methylene protons being in β-position or further in relation to double bonds, or in γ-position or further in relation to the carbonyl group. In Fig. 1, in the enlargement of the signal C of the spectrum, the peak near 1.257 ppm due to saturated acyl groups is clearly distinguishable, as it is the shoulder near 1.275 ppm of oleic acyl groups; in addition, the signal near 1.300 ppm of the oleic groups, overlaps completely with that of linoleic groups near 1.311 ppm, giving one single signal, in agreement with previous studies (Guillén & Ruiz, 2003).

In the same way, the enlargement of the signal E due to the allylic protons (α-methylenic protons in relation to only one double bond) allows us to detect the presence of oleic groups (peaks at 2.002 and 2.020 ppm) and of linoleic groups (peaks at 2.015, 2.039, 2.056, and 2.073 ppm) (Guillén & Ruiz, 2003). It should be noted that the contribution of the linolenic protons to this signal is very small due to the small proportion of these latter acyl groups in this oil, and that linoleic acyl group peaks dominate in signal E, because their proportion is higher than that of oleic (see Fig. 1).

Finally, in the signal G due to bis-allylic protons (α-methylenic protons in relation to two double bonds), the main contribution is due to the protons of linoleic acyl groups centred at 2.766 ppm, the linolenic bis-allylic protons not being appreciable, as mentioned above (see Fig. 1) (Guillén & Ruiz, 2003).

Corn oil can oxidize during storage and this provokes a decrease mainly in the concentration of unsaturated acyl groups, which are either fragmented to give compounds of lower molecular weight, or react with other acyl groups forming dimers, oligomers or polymers with different level of modifications in their chains. Obviously, all these changes cause the disappearance of some kinds of hydrogen atoms from the oil liquid phase, and the appearance of new ones, these changes being reflected in their ^{1}H NMR spectra.

In Fig. 2 the region between 0 and 5.5 ppm of some of the corn oil samples being in different oxidation levels is given, together with some enlarged signals (A + B, C and E). In this figure it can be observed that sample **C11** has a higher degradation level than **C10**, this latter sample being more oxidized than previous samples, such as **C1**. This can be deduced because the height of the signals E and G, due to allylic and bis-allylic protons respectively, become progressively smaller, in comparison with signals D and F, which are due to α- and β-methylenic protons in relation to the carbonyl groups, and are much less affected during oil oxidation.

In this figure it is also evident that the degradation suffered by these oils affects linoleic groups to a higher degree than it does oleic or saturated acyl groups. This can be observed more clearly in the enlargement of some signals, like the signal of methylic protons (A), where the height of the signal at 0.889 ppm of linoleic acyl groups becomes smaller than that of oleic plus saturated acyl groups at 0.879 ppm in the most oxidized samples (see **C11**), showing its preferential degradation. The same can be deduced from the observation of the enlarged signal C (see Fig. 2), in which the diminution of the signal near 1.302 ppm (owing to the overlapping of the signals of linoleic plus oleic groups) in comparison with the signal near 1.257 ppm (due to saturated acyl groups) and with the shoulder near 1.275 ppm (owing to oleic groups), is evident in the oxidized samples **C10** and **C11**. The preferential degradation of linoleic groups is also observable in signal E, in which the higher the oxidation level is, the lower the height of signal of linoleic groups is (mainly 2.039 and 2.056 ppm).

Simultaneously with linoleic acyl group degradation, new small signals appear in this region of the spectrum, centred at 1.55, 2.55, 2.89 and 3.10 ppm; some of these signals could be tentatively assigned to protons of mono- and di-epoxide structures (Pouchert & Behnke, 1993).

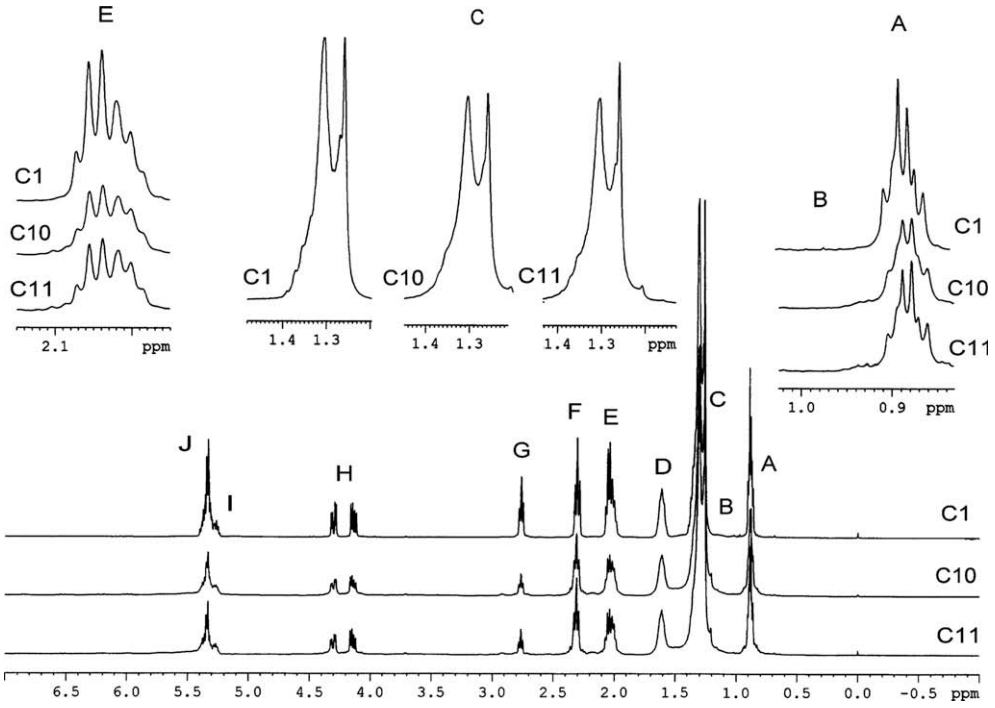

Fig. 2. Region between 0 and 5.5 ppm of the ^{1}H NMR spectra of some of the corn oil samples found in different oxidation levels, together with some suitably enlarged signals.

As a consequence of the degradation of acyl groups, new compounds are formed. As can be seen in Fig. 3, some of these produce signals in the ^{1}H NMR spectral regions between 5.4 and 7.2 ppm and between 7.4 and 10 ppm. As can be observed between 8.3 and 9.0 ppm, in the spectra of samples from **C2** to **C11** signals of protons of hydroperoxy groups are present; it should be noted that the chemical shift of the hydroperoxide group protons is higher and the signal is broader in line with the higher oxidation level of the sample. This functional group can be supported in triglyceride chains having conjugated-dienic systems or not. Fig. 4 shows, as examples, some of the possible structures of hydroperoxides formed in oxidation processes of linoleic acyl groups.

Fig. 3 also shows that the spectra of samples from **C2** to **C11** include signals of protons belonging to (*Z,E*) conjugated-dienic systems supported in chains having hydroperoxy groups; these are multiplets centred near 6.56, 6.00, 5.58 and 5.45 ppm (Neff, Frankel, & Miyashita, 1990). The four protons of the (*Z,E*) conjugated-dienic systems generate these four different signals; the first is

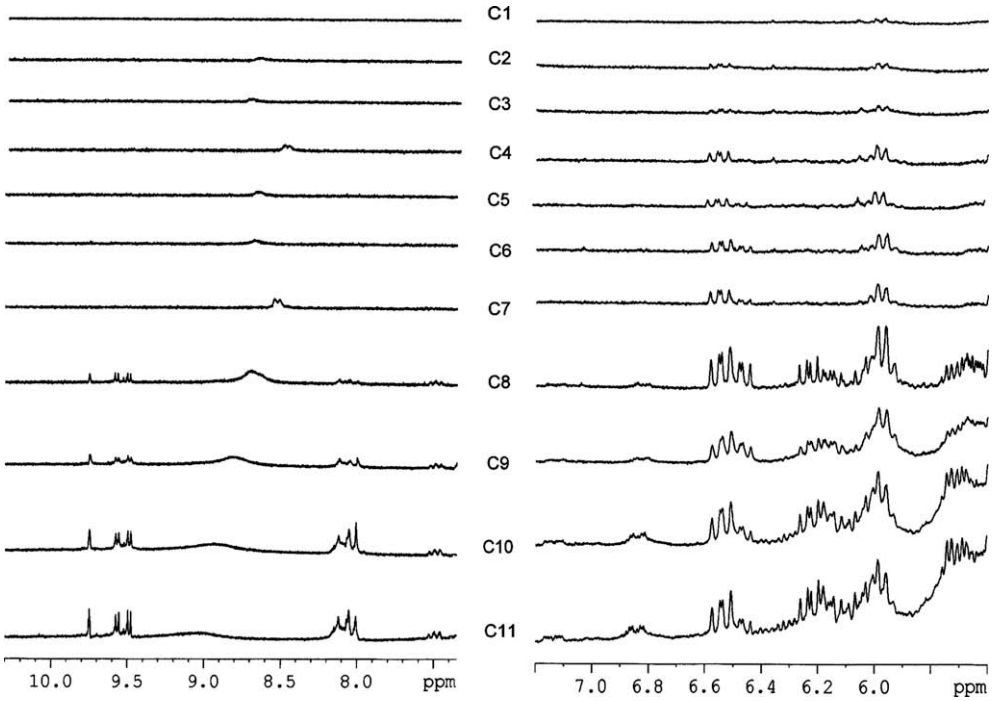

Fig. 3. Expanded regions between 7.9 and 10.3 ppm and 5.7–7.2 ppm of the ^{1}H NMR spectra of the eleven corn oil samples stored at room temperature.

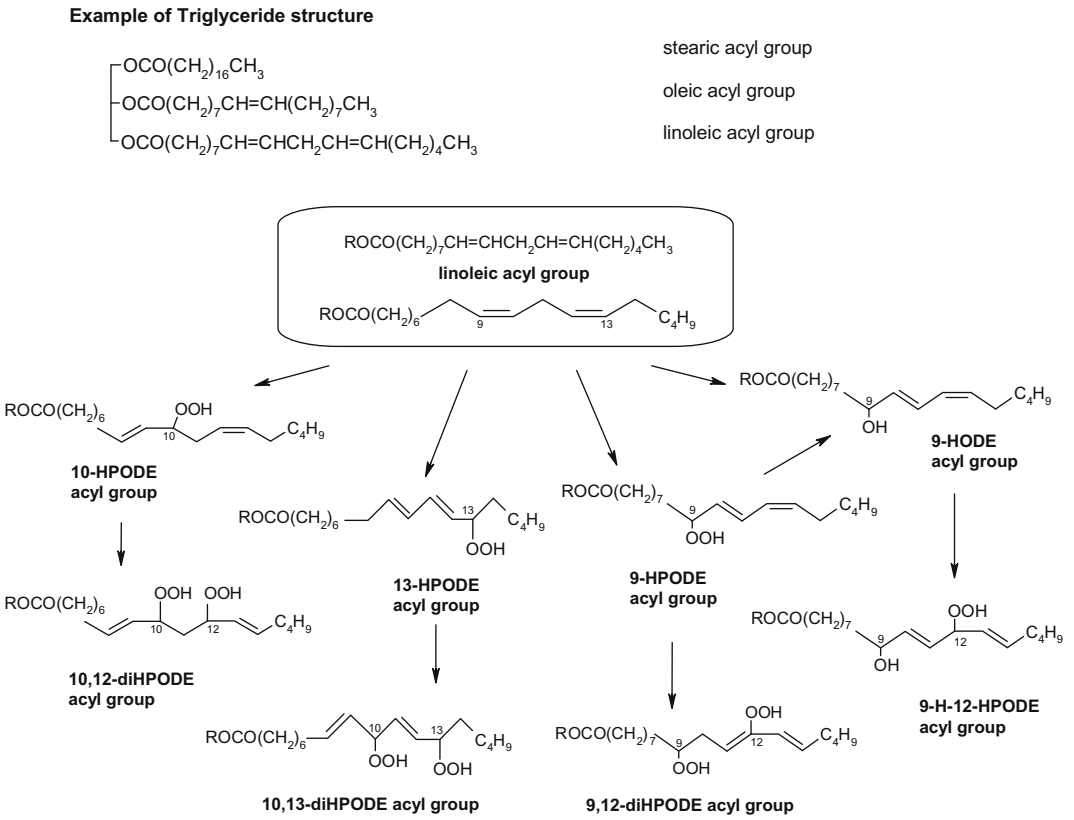

Fig. 4. Some of the many possible intermediate compounds formed in the oxidation of linoleic acyl groups: 10-hydroperoxyoctadeca-8,12-dienoic acyl group (10-HPODE), 13-hydroperoxyoctadeca-9,11-dienoic acyl group (13-HPODE), 9-hydroperoxyoctadeca-10,12-dienoic acyl group (9-HPODE), 9-hydroxyoctadeca-10,12-dienoic acyl group (9-HODE), dihydroperoxyoctadecadienoic acyl groups (10,12-, 10,13- and 9,12-diHPODE), 9-hydroxy-12-hydroperoxyoctadeca-10,13-dienoic acyl groups (9-H-12-HPODE).

the best defined of them in all samples. Fig. 4 shows, as an example, one of the possible structures of hydroperoxy-(*Z,E*)-conjugated-dienic systems (see 9-HPODE acyl group) formed in oxidation processes of linoleic acyl groups.

In addition to hydroperoxy groups and hydroperoxy-(*Z,E*)-conjugated-dienic systems, in samples from **C5** onwards hydroxy-(*Z,E*)-conjugated-dienic systems are detected. These latter systems give multiplet signals visible in the spectrum near 6.48 and 5.98 ppm (see Fig. 3), in agreement with data provided by other authors (Gardner & Weisleder, 1972). The formation of these compounds could be related to the limited amount of oxygen in contact with the oil during oxidation, in agreement with a previous study on sunflower oil (Guillén & Goicoechea, 2007). As far as we know, only two previous papers have reported the formation, in other vegetable oils after prolonged storage, of (*Z,E*) conjugated diene intermediate oxidation products having hydroxy groups (Guillén & Goicoechea, 2007; Mikolajczak, Freidinger, Smith, & Wolff, 1968). An example of one possible structure of this type is given in Fig. 4 (see 9-HODE acyl group). It could be formed directly from the linoleic acyl group or from the corresponding hydroperoxide. It should be pointed out that the hydroxides 9-HODE and 13-HODE are considered to be very good markers of lipid peroxidation *in vivo* (Spiteller & Spiteller, 1997) and are the main compounds generated in the oxidation of human low density lipoproteins (LDL), which is the precondition for atherosclerosis (Jira, Spiteller, Carson, & Schramm, 1998).

Moreover, in the ^{1}H NMR spectra of **C8** and of samples with higher oxidation level (see Fig. 3) two additional multiplet signals also appear, centred near 6.25 and 5.75 ppm, corresponding to (*E,E*) conjugated-dienic systems in chains having hydroperoxy groups (Neff et al., 1990), whose intensity increases with the oxidation level of the samples. The structure of one intermediate compound of this nature is given in Fig. 4 (see 13-HPODE acyl group).

All the intermediate oxidation compounds, which were detected in those corn oil samples oxidized at room temperature and whose possible structures are shown in Fig. 4, are in agreement with intermediate compounds considered by different authors to be involved in the oxidation mechanisms of linoleic acid and methyl linoleate under various oxidative conditions (Sun & Salomon, 2004; Schneider, Porter, & Brash, 2004; Schneider, Porter, & Brash, 2008; Schneider et al., 2005; Zhang, Sun, & Salomon, 2006).

As has been said before, ^{1}H NMR spectral data can give us molar proportions and concentrations of the functional groups associated with the intermediate oxidation compounds present in the oil sample. Table 4 gives these data related to the hydroperoxy groups present in the oil samples from **C2** to **C11**, and to the different kinds of dienic systems, that is of hydroperoxy-(*Z,E*)-conjugated-dienic systems, hydroxy-(*Z,E*)-conjugated-dienic systems and hydroperoxy-(*E,E*)-conjugated-dienic systems, except for sample **C11**. In this latter sample the concentrations of these dienic systems were not determined due to the overlapping of signals in the corresponding spectral region and to the elevation of its baseline.

In Table 4 it can be observed that the concentration of hydroperoxy groups is associated with the higher oxidation degree of the sample, except in the most oxidized sample **C11**, in which an important proportion of hydroperoxides has been degraded and converted into secondary oxidation compounds. It is also noteworthy that the concentration, in all samples, of hydroperoxy groups is higher than that of hydroperoxy-conjugated-dienic systems, as is clearly shown in Table 5. This fact indicates either the existence

Table 5
Ratios between the molar concentrations of different intermediate compounds present in corn oil samples oxidized at room temperature in closed containers.

Ratios	Samples										
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**
OOH/[OOH – (*Z*,*E*) + OOH – (*E*,*E*)]	–	5.3	3.3	1.6	1.9	1.4	1.3	1.6	1.3	1.2	n.d.
OOH–(*Z*,*E*)/OOH–(*E*,*E*)	–	–	–	–	–	–	–	3.0	1.9	1.6	n.d.
OOH/OH–(*Z*,*E*)	–	–	–	–	8.8	3.8	4.5	2.9	2.5	2.6	n.d.
OOH–(*Z*,*E*)/OH–(*Z*,*E*)	–	–	–	–	4.6	2.7	3.6	1.3	1.2	1.3	n.d.

Abbreviations: OOH, hydroperoxy groups; OOH-(*Z*,*E*), hydroperoxy-(*Z*,*E*)-conjugated-dienic systems; OH-(*Z*,*E*), hydroxy-(*Z*,*E*)-conjugated-dienic systems; OOH-(*E*,*E*), hydroperoxy-(*E*,*E*)-conjugated-dienic systems; n.d., not determined due to the overlapping of signals.

of hydroperoxy groups in acyl chains in which there are no conjugated-dienic systems (see Fig. 4, 10-HPODE acyl group, 10,12-diHPODE acyl group, 10,13-diHPODE acyl group, 9,12-diHPODE acyl group), or the existence of acyl chains having two hydroperoxy groups and one conjugated-dienic system, in agreement with intermediate compounds proposed by other authors (Schneider, Porter, & Brash, 2004, 2008; Schneider et al., 2005; Sun & Salomon, 2004; Zhang et al., 2006) for the oxidation of linoleic acid and methyl linoleate under varied oxidative conditions. Data in Table 5 indicate that in the sample with an incipient oxidation level (**C2**) the number of hydroperoxy groups in acyl chains with no conjugated double bonds is nearly five times higher than the number of hydroperoxy-conjugated-dienic systems, and this value is always higher than unity even in the most oxidized samples.

As mentioned above, hydroperoxy-(*Z*,*E*)-conjugated-dienic systems are present in all samples (from **C2** to **C11**) and their concentration is higher, in line with the higher oxidation degree of the sample. By contrast, hydroperoxy-(*E*,*E*)-conjugated-dienic systems are only present in samples in an advanced oxidation state (from **C8** to **C11**) and their concentration is also linked with the higher oxidation degree of the sample, but is always smaller than that of hydroperoxy-(*Z*,*E*)-conjugated-dienic systems, as Tables 4 and 5 show. As mentioned above, samples having intermediate and advanced oxidation level contain hydroxy-(*Z*,*E*)-conjugated-dienic systems in increasing concentration, in line with the oxidation level of the sample. However, the concentration of hydroperoxy derivatives is always higher than that of hydroxy derivatives (see Tables 4 and 5), the ratio between them being near nine in sample **C5** and near 2.5 in the most oxidized samples (**C9** and **C10**). Likewise, the concentration of hydroperoxy-(*Z*,*E*)-conjugated-dienic systems is higher than that of hydroxy-(*Z*,*E*)-conjugated-dienic systems (see Table 4), varying the ratio between the concentration of these systems from near five in sample **C5**, to a constant value near 1.3 in the most oxidized samples (**C8**, **C9** and **C10**). To the best of our knowledge, this is the first time that molar concentrations of intermediate oxidation groups are determined in oxidized oils from ^{1}H NMR data.

In addition, in the ^{1}H NMR spectra of samples in advanced oxidation states (from sample **C8** onwards) two groups of unidentified signals centred near 7.5 ppm (see Unidentified 1, Table 3) and near 8.1 ppm (see Unidentified 2, Table 3) are present. The assignment of these signals is difficult, although the second one could be due to hydroperoxy or hydroxy protons.

In samples having a certain oxidation level, not only intermediate but also secondary oxidation compounds are present. Among these latter compounds, there are aldehydes whose protons signals appear in the spectral region between 9.3 and 10 ppm, as can be observed in Fig. 5 in samples from **C8** to **C11**. The assignment of these signals is given in Table 3. The aldehydes found are: (*E*)-2-alkenals whose aldehydic proton gives a doublet signal (signal a) centred at 9.491 ppm; (*E*,*E*)-2,4-alkadienals whose aldehydic proton gives a doublet signal (signal b) centred at 9.527 ppm; alkanals whose aldehydic proton gives a triplet signal (signal f) centred at 9.748 ppm; the genotoxic and cytotoxic 4-hydroxy-(*E*)-2-alkenals

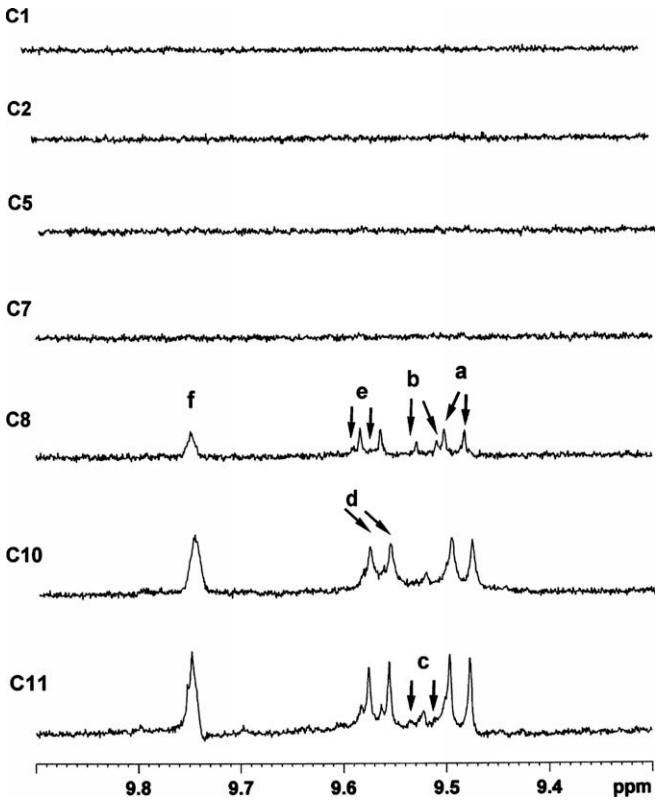

Fig. 5. Enlargement of the region between 9.3 and 10.0 ppm of the ^{1}H NMR spectra of some of the corn oil samples stored at room temperature: (a) doublet signal of (*E*)-2-alkenals, (b) doublet signal of (*E*,*E*)-2,4-alkadienals, (c) signal of 4,5-epoxy-(*E*)-2-alkenals, (d) doublet signal of 4-hydroxy-(*E*)-2-alkenals, (e) doublet signal of 4-hydroperoxy-(*E*)-2-alkenals, and (f) triplet signal of *n*-alkanals.

whose aldehydic proton gives a doublet signal (signal d) centred at 9.577 ppm, and 4-hydroperoxy-(*E*)-2-alkenals whose aldehydic proton gives a doublet signal (signal e) centred at 9.582 ppm; and finally, in the spectra of **C11** there is also a doublet signal (signal c) centred at 9.536 ppm due to the aldehydic proton of 4,5-epoxy-(*E*)-2-alkenals. It should be commented on that the hydroperoxy proton of 4-hydroperoxy-(*E*)-2-alkenals gives a signal near 8.2 ppm, included in the broad signal above named Unidentified Signal 2.

The aldehydic group is formed in the truncation of hydroperoxy acyl groups, as Fig. 6 shows, and they can be supported in small molecules or in the truncated acyl group of the triglyceride. Aldehydes of low molecular weight can remain dispersed in the oil liquid matrix or can escape to the surrounding air, producing the typical rancid odour. For these reasons, the ^{1}H NMR signals of the aldehydic protons present in these oil samples can belong to aldehydes of low molecular weight and to aldehydes present in truncated acyl groups.

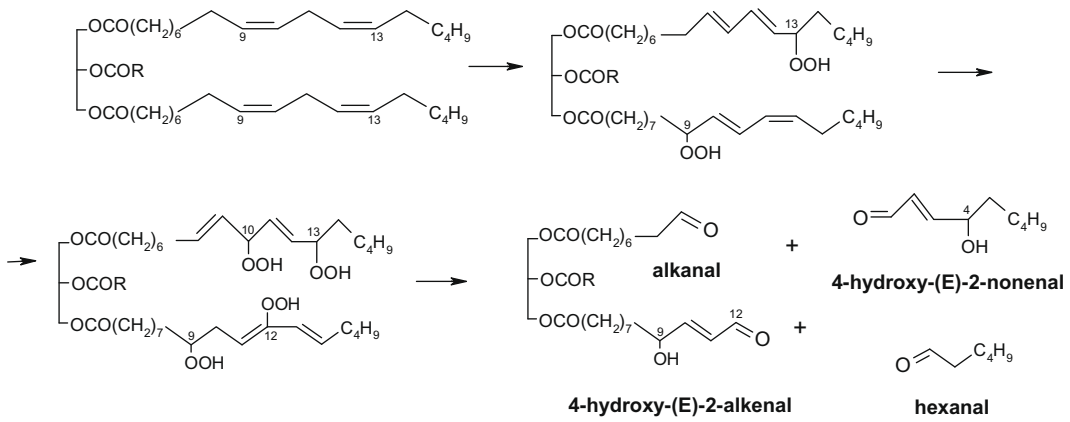

Fig. 6. Examples of some of the possible fragmentations of intermediate oxidation compounds coming from linoleic acyl groups to give secondary oxidation compounds.

Among the aldehydes detected, the γ-oxygenated α,β-unsaturated are very reactive, whether they are bonded to truncated triglycerides or constitute molecules of low molecular weight. Among these latter ones, the most known is 4-hydroxy-(*E*)-2-nonenal, whose biological activity has been a subject of great concern (Esterbauer et al., 1991; Guillén & Goicoechea, 2008; Zarkovic, 2003); however, recent studies have shown that other γ-oxygenated α,β-unsaturated aldehydes detected in this study, such as 4,5-epoxy-(*E*)-2-decenal, have similar reactivity to 4-hydroxy-(*E*)-2-nonenal (Jian, Arora, Oe, Shuvaev, & Blair, 2005). Likewise, it has been suggested that truncated 4-hydroxy-(*E*)-2-alkenal phospholipids promote the formation of foam cells, which are precursors of the atherosclerotic plaques, among other biological activities (Salomon, 2005; Sun & Salomon, 2004). The discovery of these toxic compounds in corn oil samples stored at room temperature helps to explain the results obtained by other authors in previous papers, in which after oral administration to rats of corn oil oxidized at room temperature for 48 months, tumour promotion and initiation was found in all the investigated organs (liver, lung, kidney, thymus and spleen) (Perjesi, Pinter, Gyongyi, & Ember, 2002).

Table 6 gives the relative molar proportions and concentrations of the several kinds of aldehydes. It can be observed that the concentration values of these secondary oxidation compounds in oxidized corn oil samples are inside specific ranges, showing that their formation is due to specific mechanisms and rules. So, in oxidized samples in not very advanced oxidation levels the concentration of alkanals, (*E*)-2-alkenals and 4-hydroxy-(*E*)-2-alkenals is of a very similar order (see samples **C8** and **C9**), indicating that the reactions that produce these compounds are very closely related. However, in the most oxidized samples the concentration of (*E*)-2-alkenals and 4-hydroxy-(*E*)-2-alkenals is slightly higher than that of alkanals. And the concentration of (*E,E*)-2,4-alkadienals and 4-hydroperoxy-(*E*)-2-alkenals is somewhat lower.

When comparing the ^{1}H NMR signals of the intermediate oxidation compounds and of the aldehydes present in corn oil samples here studied with those of sunflower oil samples oxidized at room temperature in closed receptacles (Guillén & Goicoechea, 2007), great similarity is found in relation to the nature and to the proportions of these kinds of compounds. This fact shows that oils with similar proportions of acyl groups submitted to these oxidative conditions evolve through similar mechanisms.

When comparing the nature and proportions of the intermediate and secondary oxidation compounds present in the corn oil here studied with those found in corn oil samples submitted to 70 °C with aeration (Guillén and Ruiz, 2005b), significant differences are found. In relation to the intermediate compounds, samples oxidized at 70 °C do not contain hydroxy-(*Z,E*)-conjugated-dienic systems, and in contrast to the samples stored at room temperature, hydroperoxy-(*Z,E*)- and hydroperoxy-(*E,E*)-conjugated-dienic systems are present in samples having low and high oxidation level, hydroperoxy-(*E,E*)-conjugated-dienic systems always being in higher concentration than hydroperoxy-(*Z,E*)-conjugated-dienic systems; these differences indicate that both processes evolve in different ways. The presence of hydroxides in samples oxidized at room temperature in closed receptacles and their absence in the process at 70 °C with aeration could be associated with the limited concentration of air in the first case. However, the presence of hydroperoxy-(*E,E*)-conjugated-dienic systems in samples having low oxidation level reached at 70 °C with aeration, and their higher proportion in relation to the hydroperoxy-(*Z,E*)-conjugated-dienic systems in all samples oxidized under these latter oxidative conditions, could be associated with the higher temperature to which these samples were submitted. Furthermore, differences related to secondary oxidation compounds are also found between corn oil samples oxidized at room temperature and at 70 °C with aeration (Guillén and Ruiz, 2005b). The main difference is in the proportions of 4-hydroperoxy and 4-hydroxy-(*E*)-alkenals; in samples oxidized at 70 °C the concentration of 4-hydroperoxy-(*E*)-2-alkenals is always higher or similar to that of 4-hydroxy-(*E*)-2-alkenals (Guillén and Ruiz, 2005b), while in the samples stored at room temperature the concentration of these latter compound groups is always higher than that of the

Table 6
Relative molar proportions (rmp) and concentrations (mmol/kg) in corn oil of different secondary oxidation compounds present in corn oil samples oxidized at room temperature in closed containers.

Compounds		Samples			
		C8	**C9**	**C10**	**C11**
Total aldehydes	rmp	0.28	0.90	1.19	1.17
	mmol/kg	15.46	49.22	65.44	64.08
Alkanals	rmp	0.06	0.22	0.28	0.27
	mmol/kg	3.66	11.96	15.13	14.69
(*E*)-2-alkenals	rmp	0.07	0.25	0.35	0.29
	mmol/kg	3.82	13.77	19.28	16.06
(*E,E*)-2,4-alkadienals	rmp	0.04	0.11	0.14	0.12
	mmol/kg	1.97	6.12	7.65	6.45
4-Hydroxy-(*E*)-2-alkenals	rmp	0.07	0.24	0.30	0.34
	mmol/kg	3.82	12.89	16.50	18.57
4-Hydroperoxy-(*E*)-2-alkenals	rmp	0.04	0.08	0.13	0.09
	mmol/kg	2.19	4.48	6.88	5.13
4,5-Epoxy-(*E*)-2-alkenals	rmp	–	–	–	0.06
	mmol/kg	–	–	–	3.17

n.d. = Not determined due to the overlapping of signals.

former. This fact could be due to either the limited concentration of oxygen in the oxidation at room temperature, which could favour the formation of hydroxy over that of hydroperoxy aldehydes, or to the presence of hydroxy-(*Z*,*E*) conjugated diene systems amongst the intermediate oxidation products, which can evolve directly to give 4-hydroxy-(*E*)-2-alkenals directly, as has been described (Schneider et al., 2004).

To the best of our knowledge, this is the first time that concentrations of several functional groups belonging to intermediate oxidation compounds as well as of some secondary oxidation compounds, such as γ-oxygenated α,β-unsaturated aldehydes present, in the oil liquid matrix of oxidized oils, have been determined from ^{1}H NMR spectral data, the subject of study being corn oil samples stored at room temperature in closed containers. This determination takes a few minutes and does not require chemical modification of the sample and provides information not only on primary but also on secondary oxidation products.

Finally, the principal component analysis of the five variables associated with oxidation conditions (OC), storage time (ST), contact surface (CS), air volume (AV), oil volume (OV) and air–oil volume ratio (AOVR), was performed, and these were reduced to two principal components called OC1 and OC2. They explain 77.6% of the total variance. The first component OC1 explains 55.3% of the total variance and is given by the expression:

$$OC1 = -0.153(ST) + 0.227(CS) - 0.214(AV) + 0.433(OV) - 0.397(AOVR).$$

The second component OC2 explains 22.3% of the total variance and is given by the expression:

$$OC2 = -0.200(ST) + 0.308(CS) + 0.769(AV) - 0.154(OV) + 0.202(AOVR).$$

Fig. 7A shows the relations obtained in this preliminary study graphically. Likewise, the principal component analysis of the two variables associated with the oxidation level (OL) of the samples, hydroperoxides (HY) and aldehydes (AL), was performed and these were reduced to one component, named OL; this principal component OL explains 91.3% of the variance and is given by the expression:

$$OL = 0.523(HY) + 0.523(AL).$$

In order to find relationships between oxidation level and oxidation conditions, multiple linear regression between the principal components OL, OC1 and OC2 was performed; the correlation coefficient ($r = 0.941$) of the equation obtained, $OL = -0.844(OC1) - 0.415(OC2)$, shows that a close relation exists between the oxidation level of the samples defined in this way and the two new variables associated to the oxidation conditions, as could be expected. It can be observed that the OC1 variable is more closely related to OL than OC2. This is in agreement with the above. Fig. 7B represents OL values obtained from the principal component analysis of HY and AL variables vs. OL predicted values from the regression equation given above. In spite of the fact that important factors, such as main and minor components and the concentration and nature of the antioxidant added to the original oils, have not been included in this statistical study, a close relationship has been found to exist between oxidation level (OL) of the samples and oxidation conditions (OC1 and OC2), presented by principal component analysis.

In short, the study of the results obtained by using ^{1}H NMR spectroscopy reveals that in corn oil samples stored at room temperature in closed receptacles there is a preferential degradation of linoleic acyl groups. As far as primary oxidation compounds are concerned, samples with low level of oxidation (**C2**–**C4**) contain hydroperoxides, a small proportion of them (*Z*,*E*) having conjugated double bonds. In samples with intermediate oxidation level, from **C5** onwards, in addition to the above mentioned hydroperoxides, signals of hydroxy derivatives having (*Z*,*E*) conjugated-dienic systems also appear. In samples whose oxidation state is advanced, hydroperoxides having (*E*,*E*) conjugated double bonds and aldehydes are also detected. Amongst aldehydes the main are alkanals, (*E*)-2-alkenals, and 4-hydroxy-(*E*)-2-alkenals; in addition, there are (*E*,*E*)-2,4-alkadienals, 4-hydroperoxy-(*E*)-2-alkenals and in very small proportions 4,5-epoxy-(*E*)-2-alkenals. These results indicate that the oxidation process that takes place at room temperature in closed receptacles is somewhat different to that provoked at 70 °C with aeration. The most important differences found between both processes refer to the nature and proportions of the intermediate compounds formed, to the timing of their formation, and to the relative proportions of the secondary oxidation products generated. It is particularly worth noting the formation of hydroxy derivatives with (*Z*,*E*) conjugated double bonds. Moreover, the same ^{1}H NMR spectra made it possible to both identify and quantify all these types of compounds formed in the oxidation process in a very simple and fast way.

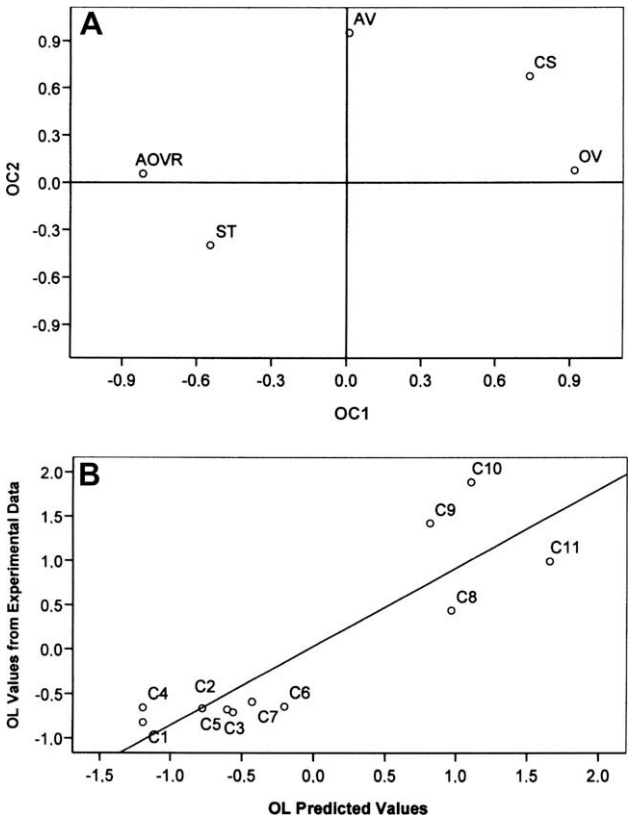

Fig. 7. (A) OC1 (55.3%) versus OC2 (22.3%) of the variables ST, CS, AV, OV and AOVR. (B) Oxidation level (OL) values defined as the principal component of variables HY and AL vs. predicted values of the oxidation level from the multiple linear regression equation of OL, OC1 and OC2 principal components.

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