Study by Proton Nuclear Magnetic Resonance of the Thermal Oxidation of Oils Rich in Oleic Acyl Groups

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ABSTRACT: The oxidation process of olive, hazelnut, and peanut oils at 70°C with circulating air has been studied by means of ^{1}H NMR. The evolution of the process, including the rate of degradation of the acyl groups and of formation and degradation of primary oxidation products, as well as the rate of formation of secondary oxidation products such as aldehydes, is commented on and compared with the oxidation of oils rich in polyunsaturated acyl groups. Differences that are due not only to the rate of the processes but also to the nature and concentration of the generated aldehydes are observed. Oils rich in monounsaturated acyl groups generate smaller amounts of the geno- and cytotoxic oxygenated aldehydes than oils rich in polyunsaturated acyl groups.

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KEY WORDS: ^{1}H nuclear magnetic resonance; olive, hazelnut, and peanut oils; oxidation process; oxygenated aldehydes; primary oxidation compounds; secondary oxidation compounds.

The compositions of dietary oils and fats vary in relation not only to the main components but also to the minor ones. These differences in composition produce differences in the behavior and performance of oils and fats during processing; in their oxidative stability and shelf life; in their metabolism by humans after consumption; and, as a consequence, in their effects on human health.

Previous studies of edible oil oxidation using FTIR spectroscopy (1,2) have shown that their oxidation rate or oxidative stability is a function of both the unsaturation degree and the content of minor antioxidant components.

Studies on the oxidation process occurring in cells and tissues have also shown different behaviors of saturated, monounsaturated, n-6 and n-3 polyunsaturated acids. In fact, oxidation of PUFA gives rise to the formation of aldehydes that are involved in some pathophysiological processes (3). Some of these aldehydes are cyto- and genotoxic and are related to diseases such as atherosclerosis, Alzheimer's disease, cancer, and cardiovascular, inflammatory, and autoimmune diseases (4–9).

Some authors have used the ratio of olefinic protons to aliphatic protons, determined directly from the proton NMR (^{1}H NMR) spectrum, to study the oxidative stability of some oils (10–12). More recently, it has been proven that the oxidation of edible oils rich in polyunsaturated acyl groups such as sesame, sunflower, and corn oils, which are rich in linoleic groups, and rapeseed, walnut, and linseed oil, rich in linolenic groups, differs not only in the rate at which each of the stages occurs but also in the proportions and, in some cases, in the nature of the aldehydes generated (13–15). Research has also shown that toxic aldehydes, such as 4-hydroperoxy-*trans*-2-alkenals, 4-hydroxy-*trans*-2-alkenals, and 4,5-epoxy-*trans*-2-alkenals, are generated in oils rich in linoleic and linolenic acyl groups when they are oxidized at 70°C with aeration (13–15).

This paper is focused on the study by ^{1}H NMR of the oxidation of edible oils rich in monounsaturated acyl groups such as hazelnut, peanut, and olive oils, the last of which is well known for its high content of natural antioxidants. The aims of this study were to obtain information about the oxidation process of these monounsaturated acyl-rich oils and to compare their behavior and the aldehydes generated with that of polyunsaturated acyl-rich oils under the same oxidative conditions.

MATERIALS AND METHODS

Samples and standards. Virgin olive, hazelnut, and peanut oils were acquired from local supermarkets. Heptanal, octanal, *trans*-2-heptenal, *trans*-2-octenal, *trans,trans*-2,4-heptadienal, *trans,trans*-2,4-nonadienal, and *trans,trans*-2,4-decadienal, from Aldrich (Milwaukee, WI); 4-hydroxy-*trans*-2-nonenal, from Merck (Whitehouse Station, NJ); and 4-hydroxy-*trans*-2-hexenal, from Cayman Chemicals (Ann Arbor, MI) were used as standard compounds. In addition, deuterated chloroform, used as solvent for the acquisition of the ^{1}H NMR spectra, and tetramethylsilane (TMS), used as internal standard, were purchased from Cortec (Paris, France).

Sample oxidation. Ten grams of oil was weighed in glass petri dishes 80 mm in diameter and 15 mm high and placed in a Selecta (Barcelona, Spain) convection oven, with circulating air, whose temperature was maintained at 70°C with a stability of ±0.5%. The petri dishes were introduced into the oven without their lids to facilitate the exposure of the sample to the circulating air. The oxidation process was carried out in duplicate and was monitored daily by ^{1}H NMR.

^{1}H NMR. 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 NMR tube. The acquisition parameters were: spectral width 5000 Hz,

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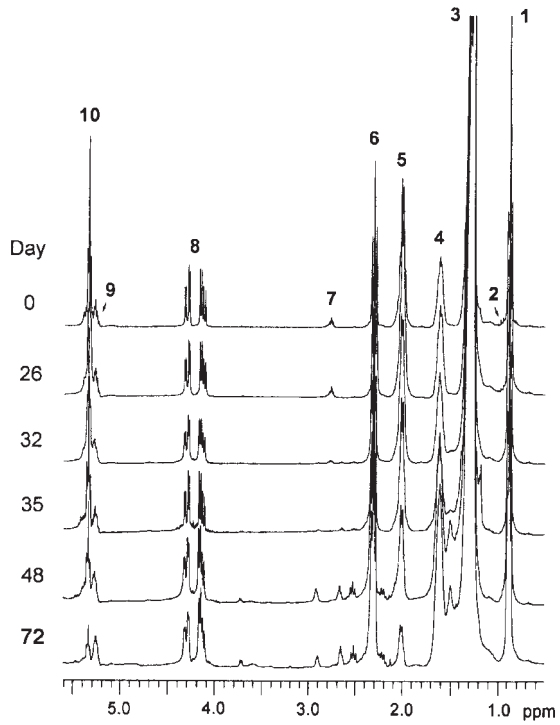

FIG. 1. Region between 0.5 and 5.5 ppm of the ^{1}H NMR spectra of virgin olive oil at different days under oxidative conditions.

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 daily throughout the oxidation process. The assignment of the signals was made as in previous studies (16–18). The area of the signal peaks was determined by using the equipment software, and the integrations were made three times to obtain average values. The ^{1}H NMR spectra, or the expanded ^{1}H NMR spectral regions, included in each figure were plotted at a fixed value of absolute intensity to be valid for comparative purposes.

RESULTS AND DISCUSSION

Typical ^{1}H NMR signals from unoxidized edible oils appear in the region between 0.5 and 5.5 ppm, as the plot for virgin olive oil in Figure 1, day 0, shows. The assignment of these signals is well known (16–19). The proportions of the different acyl groups in the virgin olive, hazelnut, and peanut oils were determined from ^{1}H NMR spectra acquired from the unoxidized oils, as described in previous papers (20,21) and as are given in Table 1. The three oils are seen to be rich sources of oleic groups, peanut oil being the richest in the linoleic group.

When these oils are subjected to oxidative conditions, they undergo changes that are detected in the ^{1}H NMR spectra. Figure 1 shows the spectral region between 0.5 and 5.5 ppm of virgin olive oil throughout its oxidation process. The rate of oxidation of virgin olive, hazelnut, and peanut oils is very slow compared with that of oils rich in polyunsaturated acyl groups (sesame, corn, sunflower, rapeseed, walnut, and linseed oils).

In Figure 1 one can see that, as the oxidation process advances, the intensity of some signals of ^{1}H NMR spectra decreases, in some cases until their disappearance. This is the case for signal **2**, which is due to the methylic protons of linolenic acyl groups; on day 26 this signal is very slight, and from this day on it is totally absent from the spectrum. In the same way, the intensity of signal **7**, due to methylenic protons in a position α to two double bonds, that is to say, due to linoleic and/or linolenic groups, also diminishes throughout the oxidation process, and the signal is not visible from day 35 onward. From these results, it is evident that a small amount of linolenic groups is present in the sample until day 26; signal **7** is only due to linoleic protons from day 27 to day 34; and no polyunsaturated group remains in the sample from day 35 onward.

The intensity of signals **5** and **10**, which are due to methylenic protons in a position α to one double bond and to olefinic protons, respectively, also decreases throughout the process, being very small at the end of the experiment, thus showing the high level of degradation undergone by the unsaturated groups.

A similar evolution is observed in the thermal degradation of hazelnut and peanut oils, but with differences in the time at which the changes occur. As hazelnut and peanut oils do not have linolenic groups in their composition, their ^{1}H NMR spectra do not have signal **2**, and signal **7** is due only to linoleic groups. In hazelnut oil spectra, signal **7** is no longer visible on day 14, and in peanut oil spectra, on day 18. Thus, observation of the ^{1}H NMR spectral region between 5.5 and 0.5 ppm provides information on the rate of degradation of not only linolenic acyl groups but also linoleic and oleic groups.

In these results, differences related to the oxidative stability of the three oils studied are evident, virgin olive oil being the most stable and hazelnut oil the least. Additionally, other factors, such as the presence of minor antioxidant components, play an important role in the oxidation rate of the oils. For example, hazelnut oil contains less linoleic and more oleic acyl groups than peanut oil, but its oxidation rate is higher, which

TABLE 1
Proportions of the Different Acyl Groups in Unoxidized Virgin Olive, Hazelnut, and Peanut Oils

	Linolenic	Linoleic	Oleic	Saturated
Virgin olive	0.7	6.0	81.3	12.0
Hazelnut	0.0	13.5	77.9	8.6
Peanut	0.0	21.6	61.8	16.6

makes it evident that differences in the oxidative stability among different oils are due not only to the structure and composition of the main components but also to minor components.

In Figure 1, total polymerization of virgin olive oil is seen to occur after 72 d; this period is shorter for peanut oil (51 d) and for hazelnut oils (20 d) (data not shown). It should be noticed that this process takes between 4 and 20 d for the oils rich in polyunsaturated acyl groups that were mentioned (13–15). As polymerization advances, the viscosity of the three oils increases, probably owing to cross-linking reactions between the chains of different TG molecules. The increasing intensity of the signal near 1.5 ppm (22) in the ^{1}H NMR spectra of virgin olive (see Fig. 1), hazelnut, and peanut oils throughout the oxidation process could be attributed to the increasing concentration of hydrogen atoms bonded to tertiary carbons; these latter can be generated in cross-linking reactions to give rise to C–C bonds between different acyl group chains, leading to polymer formation.

Other new signals appear in this region of the spectrum simultaneously with the degradation of acyl groups and the increase in viscosity. So in virgin olive (see Fig. 1), hazelnut, and peanut oil spectra, from days 32, 11, and 13 onward, respectively, signals centered at 2.52, 2.65, and 2.90 ppm are visible (see Fig. 1); some of these signals could tentatively be assigned to protons of mono- and di-epoxide structures (22). They reached a significant intensity at the end of the experiment. These signals are also visible in the ^{1}H NMR spectra of oxidized polyunsaturated oils.

Furthermore, other new signals appear at advanced oxidation stages: a double signal at 3.75 ppm, tentatively assigned to *s,n*-1,2-DAG (21,23), which is also present in the oxidized corn and sesame oil spectra but not in the spectra of any other oxidized oil rich in polyunsaturated groups; a small signal centered at 3.20 ppm, also visible in oxidized corn oil spectra; and finally, other unidentified signals, centered at 2.12, 2.20, 3.40, 3.60, 4.70, 4.90, and 5.10 ppm, that are present only in oxidized oils rich in monounsaturated acyl groups.

^{1}H NMR spectra provide information about the rate of the oil degradation and, at the same time, about the evolution of the proportions of several acyl groups throughout the oxidation process. The molar percentage of the different acyl groups in relation to the total acyl groups in the oil sample at different days of the oxidation process was determined by the introduction of the areas of certain signals in the equations previously described (20,21). The results thus obtained are represented in Figures 2A–C and show the evolution of the proportions of the different acyl groups in relation to the total acyl groups throughout the oxidation process of virgin olive, hazelnut, and peanut oils, respectively. In virgin olive oil, as Figure 2A shows, the proportions of several acyl groups remain almost unchanged until day 25, but from this day onward, a constant decrease in the proportions of linolenic, linoleic, and oleic groups is observed, in such a way that on days 28 and 41, the linolenic and linoleic groups, respectively, are totally degraded in this oil. From this day onward, the only groups that remain in the sample are oleic and saturated groups, the first ones suffering a great decrease in their proportion at the end of the experiment.

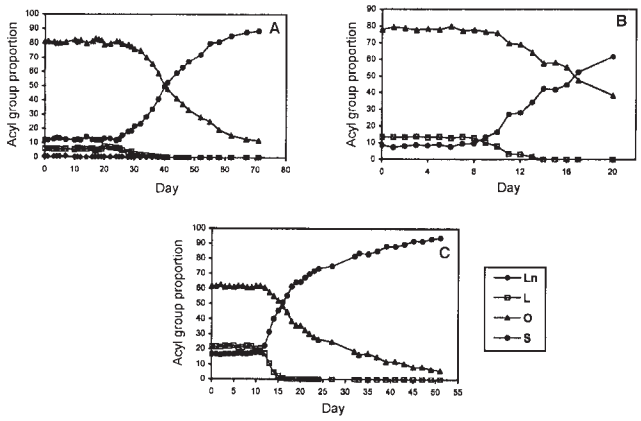

FIG. 2. Evolution of the proportions of the different acyl groups [linolenic (Ln), linoleic (L), oleic (O), and saturated (S)] throughout the oxidation process of (a) virgin olive oil; (B) hazelnut oil; (c) peanut oil.

The evolution in the proportion of acyl groups during the oxidation process of hazelnut and peanut oils is shown in Figures 2B and 2C. From day 0 to day 8 there were no significant changes in the proportions of the different acyl groups of hazelnut oil, but from day 8 onward, the proportion of linoleic groups declines until its disappearance. The proportion of oleic groups remains almost unchanged until day 10, and from this day to day 56, it diminished to half the initial proportion. Finally, as Figure 2C shows, the linoleic acyl group proportion in peanut oil remained constant until day 10, and then underwent a great decrease, being totally absent in the sample from day 16 onward; likewise, the proportion of the oleic group decreased from day 12 onward, and at the end of the experiment only a very small proportion of this acyl group remained undegraded.

In summary, in this experiment, monitored with ^{1}H NMR, great proportions of the three acyl groups were degraded; in fact, polyunsaturated groups were totally degraded long before the sample polymerized, and the proportion of oleic groups also diminished to a great extent. This contrasts with oils rich in linoleic or linolenic groups, for which the polymerization occurred much faster, and there was not enough time for total degradation of the acyl groups.

All the changes noted heretofore have been extracted from the spectral region between 0.5 and 5.5 ppm; however, other changes are also observed between 5.4 and 10.5 ppm in the ^{1}H NMR spectra. It is well known that, during oil oxidation, primary oxidation compounds or hydroperoxides supporting conjugated diene structures (24–26) are produced; hydroperoxide protons give signals in the region between 8.0 and 9.0 ppm, and protons of conjugated diene groups give signals between 5.4 and 7.0 ppm. The degradation of primary oxidation products gives rise to the generation of secondary oxidation products, among which are aldehydes that give signals between 9.2 and 9.9 ppm (16).

Figure 3 shows expanded regions of the ^{1}H NMR spectra, between 5.4 and 7.2 ppm and between 7.9 and 10.3 ppm, for virgin olive (panels a1 and a2), hazelnut (panels b1 and b2) ,

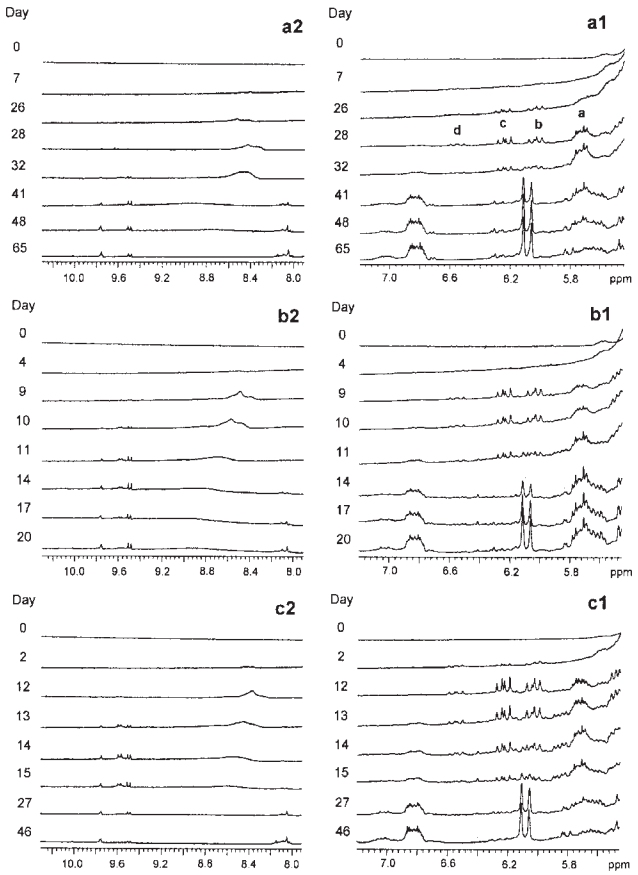

FIG. 3. Expanded regions between 7.9 and 10.3 ppm and between 5.4 and 7.2 ppm of the ^{1}H NMR spectra of (a1 and a2) virgin olive oil; (b1 and b2) hazelnut oil; (c1 and c2) peanut oil.

and peanut oils (panels c1 and c2) throughout the oxidation process. On day 0, none of the three oils showed any signal in these regions. However, on day 7 in virgin olive oil, on day 4 in hazelnut oil, and on day 2 in peanut oil, a very small signal assignable to the hydroperoxide proton, between 8.6 and 8.3 ppm, as well as incipient multiplets centered at 6.05 and 6.55 ppm (**b** and **d** in Fig. 3a1), assignable to *cis,trans*-, and at 5.70 and 6.25 ppm (**a** and **c** in Fig. 3a1), assignable to *trans,trans* conjugated double bond groups, respectively, are observed. The intensity of these signals remains constant or increases very slowly until day 26 in virgin olive oil, day 8 in hazelnut oil, and day 11 in peanut oil; on these days, signals corresponding to the hydroperoxide proton and to the conjugated diene systems are clearly observable. The intensity of these signals increases on the following days, showing the highest concentration on day 28 in virgin olive oil, on day 10 in hazelnut oil, and on day 13 in peanut oil, and from these days onward, the concentration decreases owing to their degradation. The increase of the intensity of signals due to hydroperoxide groups coincides in time with the beginning of the diminution of the proportions of linoleic groups shown in Figure 2A, 2B, and 2C, respectively.

The latter signals have also been observed in the ^{1}H NMR spectra of oxidized oils rich in linoleic and linolenic acyl groups; however, in those oils, the maximal intensity of these signals was reached much faster, showing them to have much less oxidative stability than oils rich in monounsaturated acyl groups. Moreover, the intensity of signals due to the hydroperoxide proton, as well as those of the conjugated diene systems, is higher in polyunsaturated oils than in oils rich in oleic acyl groups, probably due to their higher proportion in linoleic groups, as already noted.

Furthermore, some unidentified signals between 8.05 and 8.20 ppm and near 6.10 ppm (see Fig. 3) appear, the latter detected only in oxidized oils rich in oleic groups; both kinds of signals become visible when hydroperoxide signals, between 8.3 and 8.8 ppm, have almost disappeared and their intensity increases in more advanced stages.

In addition, on day 28 in virgin olive oil (Fig. 3a2), on day 9 in hazelnut oil (Fig. 3b2), and on day 12 in peanut oil (Fig. 3c2), incipient signals due to the aldehydic protons, between 9.4 and 9.8 ppm, and their olefinic protons at 6.82 ppm, are visible; these are secondary oxidation products generated from the degradation of hydroperoxides, and their concentration increases as that of hydroperoxides decreases. The evolution of the aldehydic signals through the oxidation process of virgin olive, hazelnut, and peanut oils can be more clearly observed in Figures 4A, 4B, and 4C, which show the enlargement of the region between 9.4 and 10.0 ppm. The following can be observed in these three cases: a double signal at 9.480 and 9.506 ppm (**e**) due to the aldehydic proton of *trans*-2-alkenals; a doublet at 9.560 and 9.586 ppm (**f**) assigned to 4-hydroxy-*trans*-2-alkenals; a double signal at 9.568 and 9.594 ppm (**g**) tentatively assigned to 4-hydroperoxy-*trans*-2-alkenals, in agreement with Sugamoto *et al*. (27) and with spectral data provided by I.A. Blair and J. Arora (unpublished data); and a small triple signal due to *n*-alkanals (centered at 9.748 ppm) (**h**).

Signals of *trans*-2-alkenals, 4-hydroperoxy-*trans*-2-alkenals, and *n*-alkanals are observed on day 28 in virgin olive oil, on day 9 in hazelnut oil, and on day 12 in peanut oil, that is to say, these are the first aldehydes detected in the oxidation process. The intensity of *trans*-2-alkenals and of *n*-alkanals increases throughout the process, whereas that of 4-hydroperoxy-*trans*-2-alkenals increases until days 35, 11, and 14 in virgin olive, hazelnut, and peanut oils, respectively, and from these days onward, it decreases until its disappearance, in some cases, such as in that of virgin olive and peanut oils.

The signal for 4-hydroxy-*trans*-2-alkenals appears on days 35, 11, and 14 in virgin olive, hazelnut, and peanut oils, respectively, that is, a few days later than the other aldehydes just discussed, and its intensity decreases throughout the oxidation process until its disappearance.

It must be pointed out that the proportions of 4-hydroperoxy- and 4-hydroxy-*trans*-2-alkenals generated are higher in peanut than in hazelnut and virgin olive oils, and this difference is closely related to their content of linoleic groups. The higher the proportion of linoleic groups in the original oil, the higher the concentration of oxygenated aldehydes generated in the oxidation process. This fact suggests that both 4-hydroperoxy- and 4-hydroxy-*trans*-2-alkenals are generated from linoleic groups.

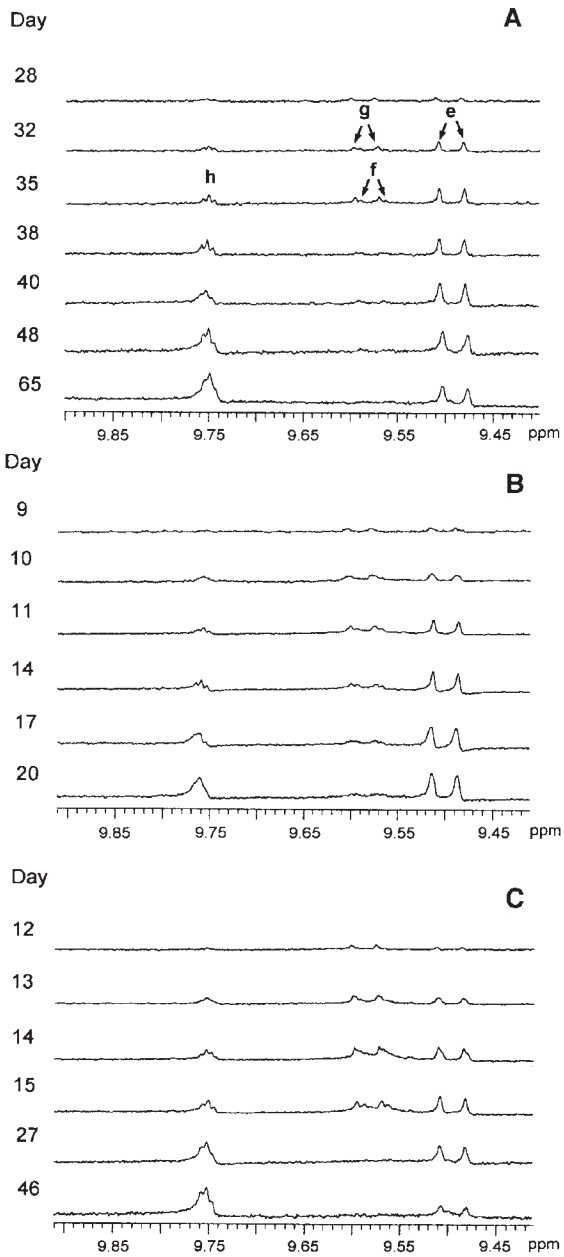

FIG. 4. Enlargement of the region between 9.4 and 9.9 ppm of the ^{1}H NMR spectra at different days of the oxidation process of (A) virgin olive oil; (B) hazelnut oil; (C) peanut oil. *e*: doublet signal of *trans*-2-alkenals; *f*: doublet signal of 4-hydroxy-*trans*-2-alkenal; *g*: doublet signal attributable to 4-hydroperoxy-*trans*-2-alkenals; and *h*: triplet signal of *n*-alkanals.

When comparing the nature, proportions, and evolution of the aldehydes generated from oils rich in oleic acyl groups with those produced from oils rich in linoleic and linolenic acyl groups, great differences are observed. Figures 5 and 6 show the expanded ^{1}H NMR spectral region, between 9.4 and 9.9 ppm, of oxidized corn and linseed oils subjected to circulating air at 70°C. In the corn oil oxidation process (Fig. 5), 4-hydroperoxy-*trans*-2-alkenals (signal **g**) and then 4-hydroxy-*trans*-2-alkenals (signal **f**) are generated in higher proportions

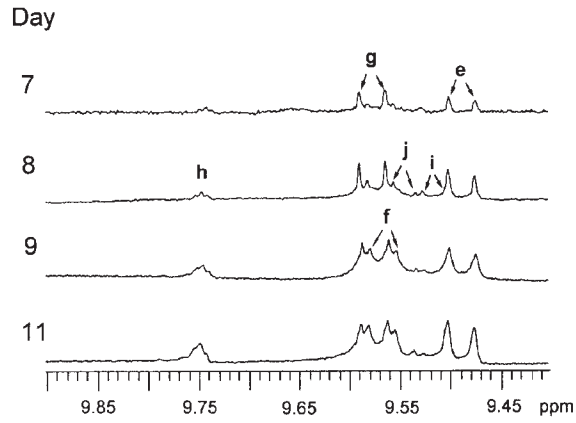

FIG. 5. Enlargement of the region between 9.4 and 9.9 ppm of the ^{1}H NMR spectra at different days of the oxidation process of corn oil. *e*: doublet signal of *trans*-2-alkenals; *f*: doublet signal of 4-hydroxy-*trans*-2-alkenal; *g*: doublet signal attributable to 4-hydroperoxy-*trans*-2-alkenals; *h*: triplet signal of *n*-alkanals; *i*: *trans,trans*-2,4-alkadienals; and *j*: 4,5-epoxy-*trans*-2-alkenals.

than *trans*-2-alkenals (signal **e**) and *n*-alkanals (signal **h**), whereas in oils rich in oleic groups the contrary occurs. Furthermore, other toxic aldehydes such as 4,5-epoxy-*trans*-2-alkenals (signal **j**) and their precursors, *trans*,*trans*-2,4-alkadienals (signal **i**) (28), which are not visible in the ^{1}H NMR spectra of oxidized oils rich in oleic groups, are also generated in the corn oil oxidation process.

Figure 6 shows that in the linseed oil oxidation process many different types of aldehydes are generated, and these give rise to an important overlapping of signals in the ^{1}H NMR region between 9.52 and 9.62 ppm. In spite of this, signals of all the previously mentioned aldehydes and of the doublet tentatively assigned to *cis,trans*-2,4-alkadienals (**k**) (at 9.580 and 9.606 ppm) can be distinguished. All these signals, between 9.52 and 9.62 ppm, show a higher intensity than those generated from oils rich in oleic groups.

Thus, in the oxidation of oils rich in oleic groups, fewer types of oxygenated aldehydes are detected, and they are in

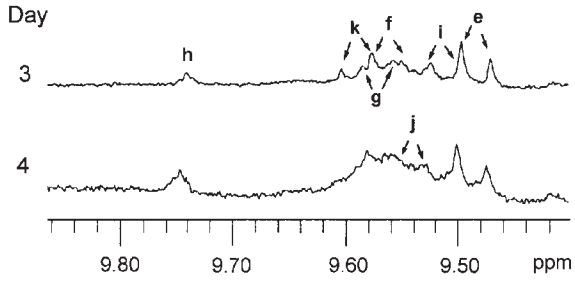

FIG. 6. Enlargement of the region between 9.4 and 9.9 ppm of the ^{1}H NMR spectra at different days of the oxidation process of linseed oil. *e*: doublet signal of *trans*-2-alkenals; *f*: doublet signal of 4-hydroxy-*trans*-2-alkenal; *g*: doublet signal attributable to 4-hydroperoxy-*trans*-2-alkenals; *h*: triplet signal of *n*-alkanals; *i*: *trans,trans*-2,4-alkadienals; *j*: 4,5-epoxy-*trans*-2-alkenals; and *k*: *cis,trans*-2,4-alkadienals.

lower concentrations, than in oils rich in polyunsaturated groups. These facts are very important because some of these aldehydes have been considered to be possible causative agents of diseases such as Alzheimer's, Parkinson's, or cancer, among others (3–9,29), and some studies have shown that these harmful compounds can be readily absorbed from the diet (30–32).

Great differences have been observed between the oxidation, with aeration, at 70°C of oils rich in oleic groups and of oils rich in polyunsaturated groups. The latter not only have less oxidative stability and polymerize faster but also generate more toxic oxygenated aldehydes than the former.

These facts should be taken into account in the different culinary practices or in the manufacture of food products, because under certain conditions, oils could yield significant proportions of toxic aldehydes.

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