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ARTICLE

Contribution to Further Understanding of the Evolution of Sunflower Oil Submitted to Frying Temperature in a Domestic Fryer: Study by ^{1}H Nuclear Magnetic Resonance

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A study is made of the evolution of the composition of sunflower oil kept over prolonged periods of time at high temperature (190 °C) in a domestic fryer. The technique used is ^{1}H NMR spectroscopy. The degradation rate of linoleic acyl groups is determined in this process, as well as the proportions of monounsaturated, of saturated plus modified acyl groups and the iodine values. Intermediate oxidation compounds having hydroperoxide groups and conjugated dienic systems were not detected; however, some secondary oxidation compounds such as aldehydes are generated very early, among them, the genotoxic and cytotoxic 4-hydroxy-*trans*-2-alkenals. Both concentrations of each kind of aldehyde at different heating times and changes in their concentration were also determined. Simultaneously, the level of oil degradation corresponding to a content of 25% of polar compounds measured by Viscofrit test was analyzed in function of the ^{1}H NMR spectra derived data.

KEYWORDS: Sunflower oil; degradation; frying temperature; deep fryer; ^{1}H nuclear magnetic resonance (^{1}H NMR); acyl groups; aldehydes; 4-hydroxy-*trans*-2-alkenals; rate of formation; viscosity; polar compounds

INTRODUCTION

Deep-frying is one of the most popular cooking methods in worldwide use due to the desirable sensory properties that fried food rapidly develops; it is very often used not only in the manufacture of many foods but also in catering and home cooking. This process involves keeping edible oils at high temperatures, in many cases, for long periods of time, either continuously or in batches; these conditions cause oil degradation and make the formation of toxic compounds possible. For this reason, the thermo-oxidative degradation of edible oils at frying temperatures has been the subject of many studies because their degradation products can be ingested with fried foods. In addition, the use of culinary oils rich in polyunsaturated instead of in saturated or monounsaturated acyl groups has created a greater awareness of oxidation processes as it is well-known that polyunsaturated acyl groups show a lower resistance to thermoxidative conditions (*1*). This being so, there are recommendations in many countries controlling frying oil use to safeguard consumer health.

Many efforts have been made to develop methods that evaluate the degradation level of frying oils and also to establish a limit past which their use is not recommended. Some of these methods are based on changes either in some of the oil physicochemical properties or in oil composition (*2*, *3*). Some of the physicochemical properties taken into account are viscosity, conductivity, and dielectric constant, among others. Some of the changes in the oil composition that are employed refer to the content of compounds such as acids, polar compounds, dimers, and polymers, among others (*4*). Nowadays, there is widespread acceptance of a method of measurement of frying oil degradation that determines the percentage of polar compounds (*5*), defined as the percentage in weight of the oil components retained on a silica gel column. Likewise, it is widely accepted that oils having a percentage in polar compounds over 25% are considered unsafe (*6*). Because the determination of this parameter is time-consuming and laborious, many efforts have also been made to develop faster methods based either on chemical reactions, on the measurement of physicochemical properties (*7*), or on the study of the equivalence of the level of degradation measured by these latter methods and the percentage of polar compounds (*6*, *8*, *9*). Some of these studies indicate that all these methods are useful and that high correlation coefficients exist between most of their results and the percentage of polar compounds; for this reason, the results provided by some of the fast methods are already expressed directly in percentages of polar compounds instead of in units of the property being measured. This is the case of Viscofrit and Testo 265 tests, which measure oil viscosity and dielectric constant, respectively.

Many efforts have also been made in the study of the chemistry of deep frying process (*10*); however, due to its complexity, to the large number of possible simultaneous reactions that can take place, and to the high number of compounds that can be formed, many aspects of this process still remain unknown. To advance knowledge of the changes produced in frying oils, this manuscript reports the monitoring by ^{1}H NMR of the evolution of the sunflower oil submitted to 190 °C in a domestic fryer. It is expected that this technique, which has proved to be a very useful tool in the study of edible oils oxidation processes provoked

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under very different conditions (*11*−*15*) will give an insight into the chemistry of the frying process. Furthermore, to the best of our knowledge, this method has not been applied to the study of oils submitted to frying conditions. The homogeneity of the degradation process at the top and at the bottom of the fryer will also be studied. At the same time, the degradation level of this oil will be monitored by the Viscofrit test to know which level of degradation measured by ^{1}H NMR data corresponds with the 25% of polar compounds measured by the Viscofrit test. Furthermore, it will be studied if the sunflower degradation process under frying conditions (without food) evolves in a homogeneous way over time or if it undergoes any significant or specific changes before or after the maximum permitted percentage of polar compounds is reached.

MATERIALS AND METHODS

Samples. Among the great variety of edible oils available, sunflower oil was selected because it is rich in polyunsaturated omega-6 acyl groups and, therefore, is highly sensitive to thermoxidative processes. The oil was purchased from a local supermarket and fits the legal requirements of the European Union for edible sunflower oils.

Heating Conditions. In each run, 2 L of sunflower oil was successively heated at 190 °C for periods of 8 h/day over 4 days for up to 32 h in a domestic deep fryer (Solac, 220−240 V, 50−60 Hz, 1800 W). The dimensions of the stainless steel tank of the fryer were 20.5 cm wide × 23.5 cm long × 13.5 cm high. Throughout the heating process no amount of oil was replenished and the cover was kept closed. The oil was stored at room temperature between the heating episodes. As mentioned above, oil samples were periodically taken in duplicate from the bottom and from the top of the stainless steel tank to evaluate the homogeneity of the degradation process in different locations of the oil tank. The temperature was periodically tested by a calibrated thermometer. When necessary, samples were refrigerated until their study by ^{1}H NMR to avoid or hinder continuation of the degradation process.

Acquisition of the ^{1}H NMR Spectra and Derived Data. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz. The oil sample (200 μL) was mixed in a 5 mm diameter tube with 400 μL of deuterated chloroform that contained 0.2% of nondeuterated chloroform and a small amount (0.03%) of tetramethylsilane (TMS) as internal reference. 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 main signals of the original sunflower oil, as well as of some of the new signals, appeared in the ^{1}H NMR spectra of sunflower oil samples having a certain degradation level was made as in previous studies (*11*, *16*) and these are given in **Tables 1** and **3**, respectively.

Compounds, such as 4-hydroxy-(*E*)-2-nonenal and 4-hydroxy-(*E*)-2-hexenal, acquired from Cayman Chemical (Ann Arbor, MI), 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 (MI, U.S.A.), were used as standard compounds for identification purposes.

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. The percentage of the linoleic, oleic, and saturated acyl groups in several samples was determined from ^{1}H NMR spectral data using two different approaches. The area of the aldehydic proton signals, which appeared as a consequence of the acyl groups degradation, was determined assigning the unity to the area of the chloroform protons signal (7.28 ppm), which has the same concentration in all ^{1}H NMR experiments as in previous studies (*15*). From these areas, the molar concentrations of these compounds were determined in the samples in which they were present, taking nondeuterated chloroform as a standard compound. All these determinations are possible because the area of the ^{1}H NMR signal is proportional to the number of protons that generate the corresponding signal. Each sample was analyzed in triplicate and data shown are average values.

Viscofrit Test. This test measures the time required to empty a determined amount of oil contained in a standard stainless steel conic receptacle through a hole in its bottom. The time required for each oil is a function of its viscosity, which in turn depends on its degradation level. However, the viscosity of unoxidized oils is a function of their composition and there are differences in viscosity between oils rich in oleic and oils rich in linoleic acyl groups; for this reason, this apparatus has been calibrated separately for these two kinds of oils. In addition, as viscosity depends not only on the type of oil and its level of degradation but also on the temperature at which the measurement is taken, calibrations between the oil temperature, time of cone emptying, and type of oil have been carried out to establish the time-length of cone emptying after which the oil should be discarded; this limit has been established for a level of degradation equivalent to 25% of polar compounds. A recent study on the results of different fast tests to evaluate the level of degradation of frying oils and percentage in weight of polar compounds has proved this equivalence (*9*).

The evaluation of the oil degradation level with this test was carried out, in duplicate, at room temperature, just before the daily heating cycle started, because the determinations should be carried out at temperatures lower than 50 °C.

Table 1. Assignment of the Signals of Sunflower Oil ^{1}H NMR Spectra[a]

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

[a] The signal letter agrees with those in **Figures 1** and **2**.

Statistic and Kinetic Studies. The degradation rate of linoleic acyl groups, as a consequence of heating, was inferred from the equation obtained by fitting the percentage of linoleic acyl groups in the oil and the time during which the oil was submitted to frying temperature; the statistical package SPSS (Inc. Chicago, 2004) was used for this purpose. Likewise, equations that relate the percentage of monounsaturated or of saturated plus modified acyl groups, as well as the iodine value with the heating time, were also obtained by using the same software package above-mentioned. By the same token, equations that relate concentration of total aldehydes or of different kinds of aldehydes and heating time were obtained; in addition, the rate of the change of the concentration of the several types of aldehydes in the oil−liquid matrix with the heating time was determined from these latter equations.

RESULTS AND DISCUSSION

As is well-known, the ^{1}H NMR spectra of unoxidized vegetable oils free of linolenic acyl groups, such as sunflower, have nine main signals between 0 and 5.5 ppm whose intensities depend on the oil composition. **Figure 1** gives the spectrum of the sunflower oil subject of study before to be submitted to frying conditions (0 h); each one of these signals are produced by the different types of hydrogen atoms present in the oil and their assignments are given in **Table 1**, in agreement with previous studies (*17*−*20*). The enlargement of some of these signals, also given in **Figure 1**, permits one to observe some features of the composition of this oil. Signal A is due to the overlapping of the triplet of methylic protons of the linoleic acyl group centered at 0.889 ppm with those of the oleic plus saturated acyl groups centered at 0.879 ppm (see **Figure 1**); this infers a higher proportion of linoleic than of oleic plus saturated acyl groups because of the higher intensity of the peak at 0.889 ppm than of the peak at 0.879 ppm, in agreement with previous results (*21*).

Signal B is due to the methylene protons in the β-position, or further in relation to double bonds, or in the γ-position, or further

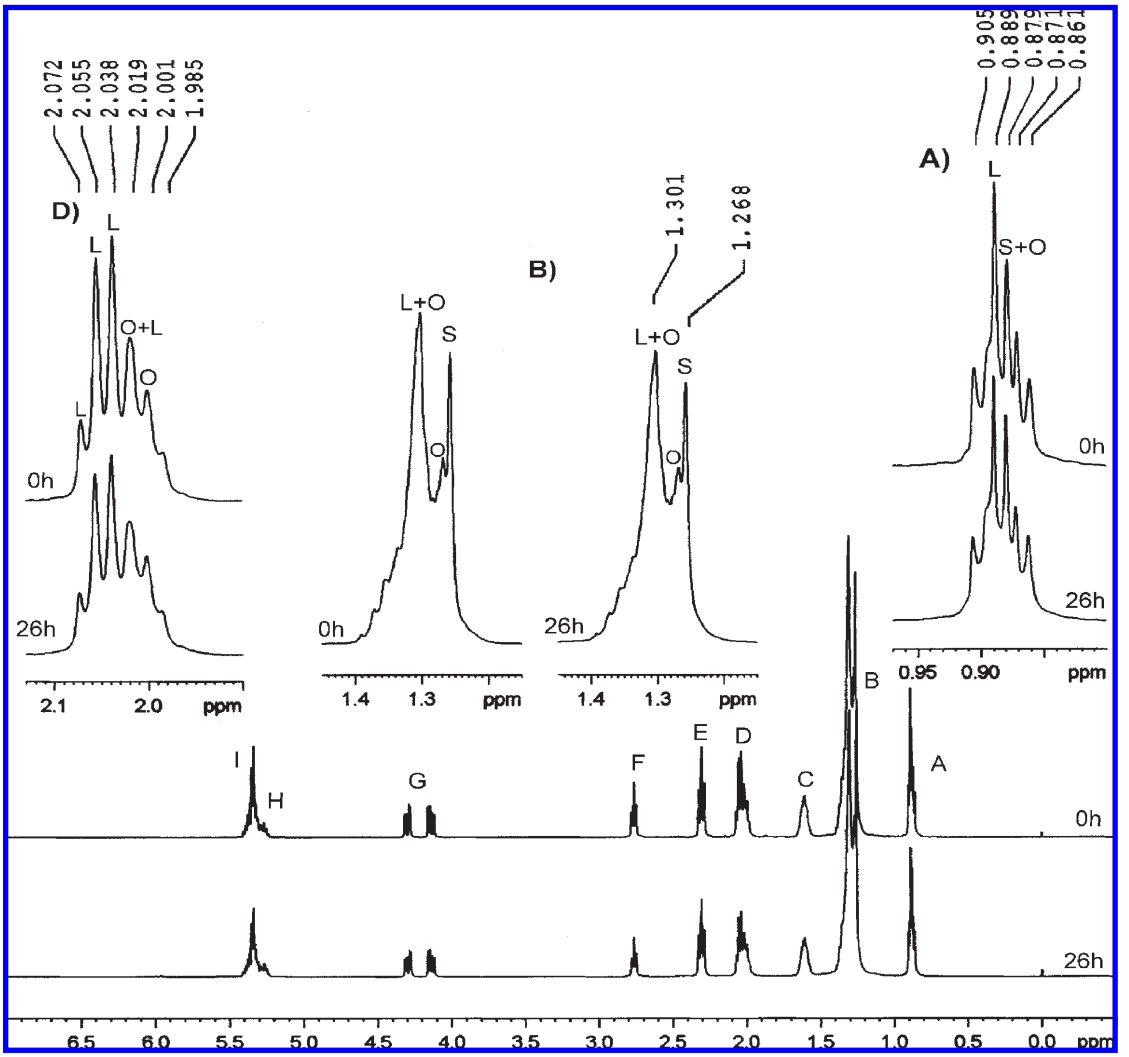

Figure 1. ^{1}H NMR spectra of the sunflower oil after 0 and 26 h of heating at frying temperature and the enlargement of some of the signals.

in relation to the carboxyl group of linoleic, oleic, and saturated acyl groups. The enlargement of this signal, given in **Figure 1**, permits one to clearly distinguish the peak near 1.268 ppm due to saturated acyl groups, the shoulder near 1.275 ppm and the peak near 1.301 ppm, both of the oleic groups, with the second overlapping with that of linoleic groups near 1.311 ppm, in agreement with previous studies (*21*).

In the same way, the enlargement of signal D due to the allylic protons (α-methylenic protons in relation to only one double bond) makes it possible to detect the presence of oleic (peaks at 2.001 and 2.019 ppm) and linoleic groups (peaks at 2.015, 2.038, 2.055, 2.072 ppm) (*21*). Signal E is due to methylenic protons in the α-position in relation the carboxyl group, and signal F is a triplet due to bis-allylic protons (α-methylenic protons in relation to two double bonds) of the linoleic acyl groups. Furthermore, the protons in positions 1 and 3 of the glyceryl structure give signal G and those in position 2 give signal H. Finally, olefinic protons give signal I.

In summary, the ^{1}H NMR spectrum of the oil gives a great deal of qualitative and quantitative information related to the different acyl groups from which the determination of their percentages is possible. One such approach can be deduced from the above-mentioned considerations by taking into account that each acyl chain contains two methylenic protons in α-position in relation to the carboxyl group (*20*, *21*). The areas of the spectrum signals D, E, and F in **Figure 1** are involved in the equations that allow the determination of these percentages. These equations are the following:

$$\text{linoleic}\ (\%) = 100(A_F/A_E) \quad (1)$$

$$\text{oleic}\ (\%) = 100[(A_D/2A_E) - (A_F/A_E)] \quad (2)$$

$$\text{saturated}\ (\%) = 100[1 - (A_D/2A_E)] \quad (3)$$

The molar percentage of the several acyl groups of the original sunflower oil determined using this approach is linoleic (L) 55.4%, oleic (O) 35.0%, and saturated (S) 9.6%.

Another approach used to determine the percentages of these acyl groups from ^{1}H NMR spectral data takes into account that mono- and diglyceride levels are low in sunflower oil and that most of the acyl chains are bonded to triglyceride structures. In this approach, the areas of signals D, F, and G of the spectrum, in **Figure 1**, are involved in the equations that permits one to determine the molar percentage of the several acyl groups, which are the following:

$$\text{linoleic}\ (\%) = 100(2A_F/3A_G) \quad (4)$$

$$\text{oleic}\ (\%) = 100(A_D - 2A_F)/3A_G \quad (5)$$

$$\text{saturated}\ (\%) = 100[1 - (A_D/3A_G)] \quad (6)$$

The molar percentages of the acyl group chains of the original sunflower oil determined using this second approach (linoleic (L)

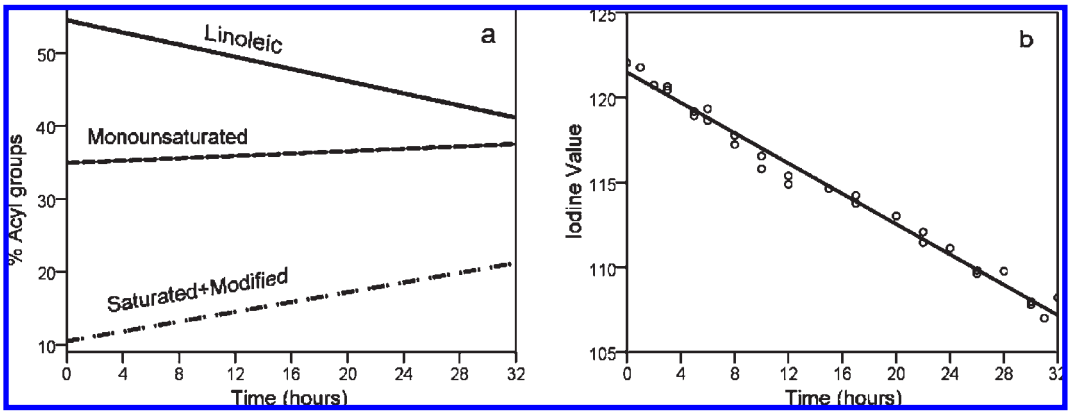

Figure 2. (**a**) Experimental values of the percentage of linoleic, monounsaturated, and saturated plus modified acyl groups vs heating time in sunflower oil at frying temperatures and the lines of the corresponding equations given in **Table 2**. (**b**) Experimental values of the iodine value vs heating time in sunflower oil at frying temperatures and the line of the corresponding equation in **Table 2**.

54.0%, oleic (O) 34.0%, and saturated (S) 12.0%) are very close to that obtained using the first one. To the best of our knowledge, this is the first time that this second approach has been proposed for the determination of the percentage of linoleic, oleic, and saturated acyl groups in oils having these kinds of acyl groups.

This oil was submitted to 190 °C in a domestic fryer for periods of 8 h per day over four days; the evolution of the oil was monitored by ^{1}H NMR. **Figure 1** gives the spectrum of the oil at two different times of heating, 0 and 26 h, respectively; it shows that the degradation of this oil reduces the intensity of the signals of the bis-allylic (signal F) and allylic (signal D) protons in relation to that of the methylenic protons in α-position (signal E) or β-position (signal C) in relation to the carboxyl group.

Furthermore, **Figure 1** also shows the enlargement of signals A, B, and D of these two spectra. The enlargement of signal A shows the decreasing intensity of the triplet due to methylic protons of linoleic in relation to that of oleic, plus saturated acyl groups as the heating time increases. When enlarged, signal B also shows a decrease with heating time in the intensity of the signal of methylene protons of linoleic, in relation to that of oleic and saturated groups. Finally, enlarging signal D of allylic protons also shows a decrease with heating in the intensity of the allylic protons signals of linoleic groups in relation to that of oleic or monounsaturated groups. All these results indicate that degradation provoked by heating tends to affect mainly the linoleic acyl groups. However, it should be mentioned that the decrease in the intensity of the above-mentioned signals in the time studied (32 h) is much smaller than that previously observed in the oxidation of this oil provoked at 70 °C with aeration (*11*) for 12 days, during which the oil was totally polymerized and the intensity of the signals due to protons of the linoleic groups was reduced considerably.

In addition to the above-mentioned, quantitative information about the degradation of the sunflower oil submitted to the frying conditions (without food) can be extracted from ^{1}H NMR spectral data. The acyl group chains with the heating may either break, giving rise to molecules of low molecular weight plus truncated acyl groups, or they may bond to other acyl chains, giving rise to dimers, oligomers, and polymers; in both cases, the acyl group chains persist and maintain the methylene group in the α-position in relation to the carboxyl group. Hydrolysis processes are not produced due to the absence of water in the system. Given the above, eq 1, representing the ratio between the number of acyl chains of linoleic acyl groups and the total number of acyl chains (modified plus unmodified), can be used to determine the molar percentage of linoleic acyl groups (L) of sunflower oil having

Table 2. Coefficients of the Equations That Relate the Molar Percentage of Several Acyl Groups [L, MU, or (S + M) % = $a + bT$] or Iodine Value [IV = $a + bT$] and Heating Time T (Hours), Their Correlations Coefficients, and the Number of Experimental Data

acyl group[a] (%) or IV	equation	a	b	R	n
L (%)	7	54.461	−0.417	0.993	30
MU (%)	8	34.917	0.081	0.923	30
S + M (%)	9	10.494	0.335	0.985	30
IV	10	121.438	−0.442	0.993	30

[a] L: linoleic acyl groups; MU: monounsaturated acyl groups; (S+M): saturated+modified.

different levels of degradation. The total number of acyl group chains (modified plus unmodified) remains constant in the oil throughout the heating process due to the absence of hydrolytic reactions. The molar percentage (%) of linoleic groups, determined in this way, was represented versus heating time (T) in **Figure 2a**; as this figure shows, this percentage decreases continuously with heating time. This decrease could be due to different causes such as the formation of carbon−carbon bonds between linoleic acyl groups to give dimers or oligomers losing one or two double bonds or the breakdown of the linoleic acyl groups to give truncated acyl groups and molecules of low molecular weight, either oxygenated or not. Both processes have been said to occur in the heating of linoleic groups at frying temperatures (*10*).

The molar percentage of linoleic groups (L) and heating time (T) data fit to a linear equation [L (%) = $a + bT$] with high correlation coefficients; both the equation and the correlation coefficients are given in **Table 2**; these results indicate that the linoleic groups degradation progresses in a linear way with heating time. The rate of degradation of linoleic groups can also be determined from derivation of eq 7 in **Table 2** in relation to heating time [$dL/dT = -0.417$]; this result indicates that, in the conditions of this study, for every hour of heating, the molar percentage in linoleic groups decreases 0.417%. From this equation it is also possible to predict the percentage of linoleic groups present in the oil at any heating time.

Likewise, eq 2 can be used to determine the molar percentage of monounsaturated acyl groups (MU) in relation to the total number of acyl groups (modified plus unmodified) in sunflower oil being in different degradation level; as it has been commented on above, the total number of acyl groups is the same in the original and in the degraded oil because the absence of hydrolytic processes during the heating. Monounsaturated acyl groups

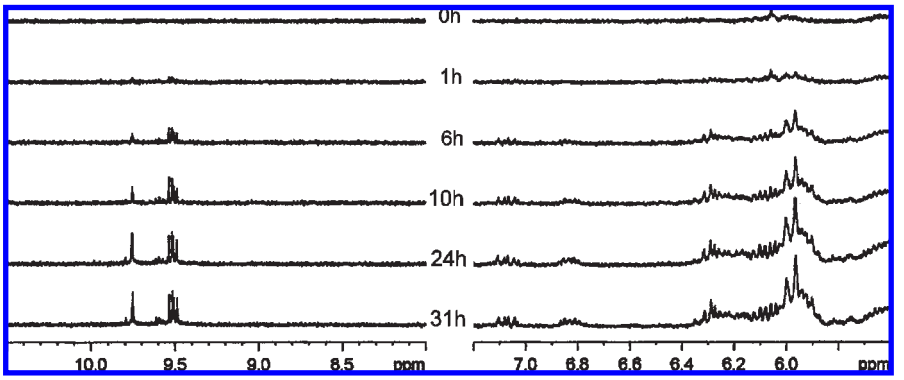

Figure 3. ^{1}H NMR spectral regions, comprised between 5.6 and 7.2 ppm and between 8.0 and 10.0 ppm, of the sunflower oil submitted at frying temperature in a domestic deep fryer during different periods of time expressed in hours.

include not only oleic but also other monounsaturated acyl groups formed from linoleic acyl groups as a consequence of the formation of carbon−carbon linkages. The molar percentage (%) of these monounsaturated MU acyl groups, determined in this way, is also represented versus heating time (T) in **Figure 2a**. As can be observed, the percentage of MU tends to increase slightly in the heating time studied. This fact can only be explained if linoleic degradation, which brings about the formation of monounsaturated acyl groups, is of greater extent than oleic degradation. This fact is in agreement with and reinforces the pathway proposed by some authors for the formation of dimers and polymers from linoleic acyl groups (*22*), which involves the formation of carbon−carbon linkages with one double bond disappearing in linoleic groups.

The molar percentage of monounsaturated acyl groups and heating time data also fit to a linear equation [$MU\% = a + bT$] with a high correlation coefficient; the equation and the correlation coefficients are also given in **Table 2**. The rate of change of the concentration of monounsaturated acyl groups with the heating time can be determined from derivation of eq 8 in **Table 2** in relation to heating time [$(dMU/dT = +0.081$]; this result indicates that, in the conditions of this study, every hour of heating provokes an increase in the molar percentage of monounsaturated acyl groups of 0.08%, it being thus possible to predict from eq 8 the percentage of monounsaturated groups present in the oil throughout the heating time. These results also indicate that, in the heating time studied, the extent of the degradation of linoleic groups to give monounsaturated acyl groups is higher than that of the degradation of oleic groups, in agreement with the above-mentioned.

From eq 3 it is also possible to determine throughout the heating time the molar percentage of saturated (S) groups together with that of other modified (M) acyl groups different from the monounsaturated acyl groups; as above, this percentage is referred to the total number of acyl groups, which remain constant throughout the heating, due to the absence of hydrolytic processes. **Figure 2a** represents this molar percentage versus time, too. These data also fit to a linear equation [$(S + M)\% = a + bT$] with high correlation coefficients; both correlation and equation coefficients are given in **Table 2**. The results obtained indicate that the percentage of S + M increases with time (0.335% each hour of heating) and this could be attributed, mainly, to the growth in percentage of modified acyl groups.

All these determinations were also made using eqs 4−6, with very similar results to those previously mentioned, maintaining the small differences found in the determination of the percentage of acyl groups of the original sunflower oil confirming their validity. These results reinforce the fact that hydrolysis does not occur in this heating process, which is consistent with the absence of water in the system and is corroborated by the small and constant intensity of the signals of mono or of diacylglycerols in the ^{1}H NMR spectra of the oil throughout the heating.

The disappearance of double bonds with the heating time in the oil should be reflected in its iodine value because this parameter informs about the unsaturation degree of the oil. In a previous study (*23*) it was shown that the iodine value of an oil can be determined by the equation IV = 10.54 + 13.39OP, where OP is the percentage of olefinic protons of the oil, which can be obtained directly from ^{1}H NMR spectral data. Using this equation, the iodine values of the original oil and of the oil after different periods of heating were determined and represented versus heating time in **Figure 2b**. It can be observed that these values fit well to a linear equation ($IV = a + bT$). Both equation and correlation coefficients are also given in **Table 2**. These results show that with heating the proportion of olefinic protons in the oil diminishes as expected, and this is reflected in turn by the reduction of its IV. In fact, for every hour of heating the IV diminishes by a factor of 0.442, which is very close to but somewhat higher than the rate of degradation of linoleic groups (0.417). Some authors (*4*) have studied the evolution of the iodine value, determined by chemical methods, of some oils with the heating time at frying temperatures and their results are in good agreement with the here obtained.

The degradation of the acyl groups of the sunflower triglycerides has as a consequence the formation of new compounds. Some of these have protons whose signals can be observed in the spectral ^{1}H NMR region comprised between 5.5 and 10 ppm. Previous studies using ^{1}H NMR spectroscopy on the degradation of edible oils submitted to several degradative conditions have shown the formation of intermediate compounds having groups such as hydroperoxy derivatives, *cis*,*trans*, or *trans*,*trans* conjugated dienic systems (*11*−*13*, *15*, *16*) and in other cases hydroxy derivatives having *cis*,*trans* conjugated dienic systems (*15*). These kinds of intermediate compounds have been described by some authors (*10*) as perhaps being formed during the heating at frying temperatures. However, as can be observed in **Figure 3**, the ^{1}H NMR spectra of the sunflower oil, submitted to the conditions of this study for 32 h in the domestic fryer, contain neither protons of hydroperoxy groups nor protons of conjugated dienic systems bonded to hydroperoxy or hydroxy derivatives; this fact indicates that, if compounds having these functional groups are formed in the sunflower oil heated at 190 °C in the fryer, they degrade at very high rates and do not accumulate in sufficient amounts to be detected by the ^{1}H NMR equipment used in this study. Nevertheless, it should be commented on that these results are in agreement with that obtained in a previous study (*24*) in which

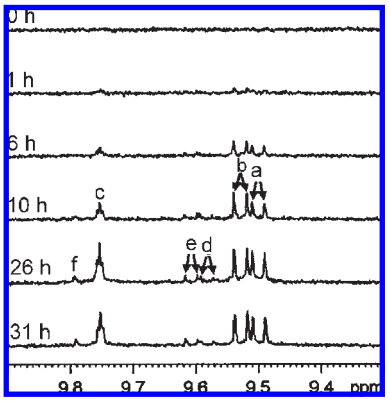

Figure 4. Enlargement of the region between 9.3 and 9.9 ppm of the ^{1}H NMR spectra of sunflower oil samples submitted at 190 °C during different periods of time in a domestic fryer. Signals: (a) doublet of (*E*)-2-alkenals; (b) doublet of (*E*,*E*)-2,4-alkadienals; (c) triplet of *n*-alkanals; (d) doublet of 4-hydroxy-(*E*)-2-alkenals; (e) doublet of (*Z*,*E*)-2,4-alkadienals; (f) triplet of 4-oxoalkanals.

no significant changes in the peroxide values of several oils submitted during prolonged periods of time to frying temperatures were observed.

The monitoring, by ^{1}H NMR spectroscopy, of several degradation processes of edible oils submitted to different degradative conditions, has shown that the intermediate compounds abovementioned evolve, leading to the formation of secondary compounds, among which are aldehydes. As **Figure 3** shows, the ^{1}H NMR spectra of the sunflower oil submitted to frying temperature has signals of protons of conjugated dienic systems of aldehydes and of other secondary oxidation products (region between 5.6 and 7.2 ppm), as well as signals of aldehydic protons (region between 9.3 and 10.0 ppm) (*11*, *25*, *26*). Although intermediate degradation compounds were not found in the sunflower oil here studied, as **Figures 3** and **4** show, the formation of aldehydes was detected from the first hour of heating. This does not mean that the formation of these compounds is not produced within shorter periods than 1 h. However, as the samples were taken every hour, the exact time at which aldehydes were formed cannot be specified. In fact, in a previous study of thermodegradation of a very small amount of oil (10 g) at 190 °C with aeration the formation of aldehydes from sunflower oil was detected by ^{1}H NMR after 15 min of treatment (*14*); furthermore, studies carried out in our laboratory (*27*) in very similar experiments (4 L of the same sunflower oil, industrial fryer, 190 °C) to the here considered, but using another technique, have shown the formation of volatile aldehydes, among which is 4-hydroxy-(*E*)-2-nonenal, in the first minute of sunflower oil heating. Likewise, other authors (*28*) have also detected in soybean oil (5 g of oil, 185 °C) after 1 h of heating the presence of 4-hydroxy-(*E*)-2-alkenals.

Figure 4 shows the enlargement of the ^{1}H NMR spectral region between 9.3 and 9.9 ppm in which the signals of the several kinds of aldehydic protons appear. Among the aldehydes detected there are *n*-alkanals, (*E*)-2-alkenals, (*E*,*E*)-2,4-alkadienals, 4-hydroxy-(*E*)-2-alkenals, (*Z*,*E*)-2,4-alkadienals, and 4-oxo-alkanals; these latter were identified in agreement with the data given by Takeoka et al. (*29*). These aldehydic groups could be supported in molecules of low molecular weight or in truncated acyl groups taking into account the several possible oxidation mechanisms previously proposed by different authors (*30*−*37*). The assignment of the ^{1}H NMR signals of these aldehydes is given in **Table 3**. As **Figure 4** shows, *n*-alkanals, (*E*)-2-alkenals, and (*E*,*E*)-2,4-alkadienals are the aldehydes that first give signals that are clearly distinguishable from the spectrum noise, whereas the signals of (*Z*,*E*)-2,4-alkadienals, 4-hydroxy-(*E*)-2-alkenals, and 4-oxo-alkanals appear as clearly distinguishable from the spectrum noise only after some hours of thermal treatment (near 3 h for (*Z*,*E*)-2,4-alkadienals, near 6 h for 4-hydroxy-(*E*)-2-alkenals, and near 9 h for 4-oxo-alkanals); this indicates that the aldehydes in the second group are formed in smaller amounts than the first and only give enough signals to be integrated after a certain accumulation; in other words, the reactions that generate the first groups of aldehydes are more favored than those that generate the second ones. It is noteworthy the presence of 4-hydroxy-(*E*)-2-alkenals because these genotoxic and cytotoxic compounds are being considered, in the last time, potential causal agents of different diseases such as Alzheimer, Parkinson, cancer, and so on (*38*).

Table 3. Chemical Shift Assignment of the ^{1}H NMR Signals of Some Secondary Degradation Products Generated from Sunflower Oil at 190 °C in a Domestic Fryer Together with Their Multiplicities[a]

signal	compounds	chemical shift[b]	functional group
a	(*E*)-2-alkenals	9.493 (d)	CHO− (aldehydic group)
b	(*E*,*E*)-2,4-alkadienals	9.520 (d)	CHO− (aldehydic group)
c	*n*-alkanals	9.748 (t)	CHO− (aldehydic group)
d	4-hydroxy-(*E*)-2-alkenals	9.573 (d)	CHO− (aldehydic group)
e	(*Z*,*E*)-2,4-alkadienals	9.593 (d)	CHO− (aldehydic group)
f	4-oxoalkanals	9.780 (t)	CHO− (aldehydic group)

[a] The signal letters agree with those of **Figures 4** and **5**. [b] d: doublet; t: triplet.

Table 4. Coefficients of the Equations [Al (mmol/L) = $aT + bT^2$] That Relate the Molar Concentration of Several Kinds of Aldehydes Al (mmol/L) and Heating Time *T* (Hours), Their Correlations Coefficients, *R*, and the Number, *n*, of Experimental Data

group of compounds	equation	*a*	*b*	*R*	*n*
total aldehydes	11	1.8925	−0.0307	0.998	27
(*E*)-2-alkenals	12	0.4647	−0.0069	0.996	30
(*E*,*E*)-2,4-alkadienals	13	0.6981	−0.0146	0.996	30
n-alkanals	14	0.4434	−0.0053	0.996	30
4-hydroxy-(*E*)-2-alkenals	15	0.0995	−0.0014	0.990	10
(*Z*,*E*)-2,4-alkadienals	16	0.1576	−0.0033	0.996	10
4-oxo-alkanals	17	0.0616	0.0000	0.986	27

These aldehydic groups could be supported in molecules of low molecular weight or in truncated acyl groups; it should be mentioned that a certain proportion of the aldehydes of low molecular weight could have escaped into the atmosphere and that ^{1}H NMR spectroscopy detects all those aldehydic groups present in the liquid phase, either of low or high molecular weight.

As mentioned before, the relative molar proportions of total aldehydes and of each kind of aldehyde, as well as their concentration at each heating interval, can be determined from the area of the ^{1}H NMR aldehydic proton signals by taking nondeuterated chloroform as the standard compound. Both sets of data, concentration of aldehydes (Al) (either total concentration or concentrations of the various groups of aldehydes abovementioned) and heating time (T), fit to parabolic equations (Al (mmol/L) = $aT + bT^2$) with high correlations coefficients. The equation and correlation coefficients, together with the number of experimental points are given in **Table 4**. **Figures 5** and **6** represent the experimental points and the lines corresponding to equations given in **Table 4**. From the observation of these figures and of the coefficients of equations in **Table 4** it is evident that at the beginning of the heating the concentration of total aldehydes, of alkanals, (*E*)-2-alkenals, and of (*E*,*E*)-2,4-alkadienals increases almost linearly with the time; however, as the heating time increases, the evolution of the concentration of these

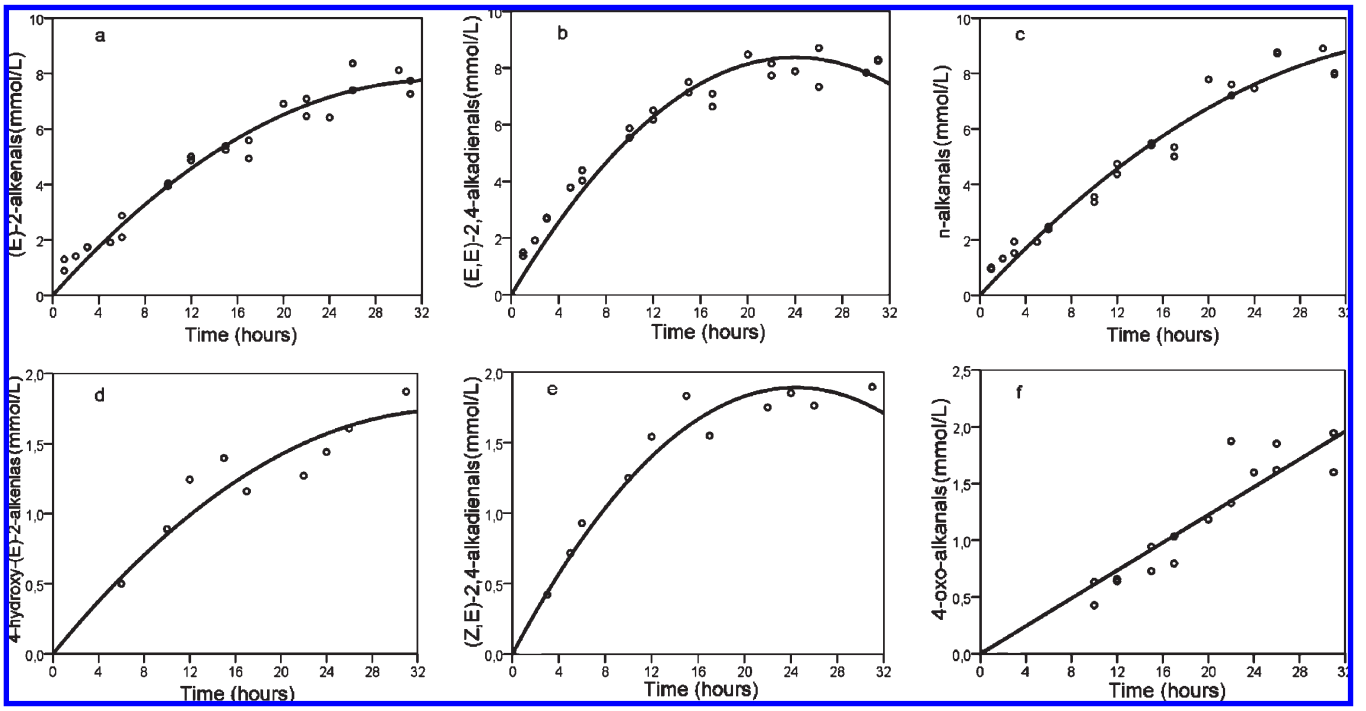

Figure 5. Experimental values of the concentration in the oil of the different kinds of aldehydes expressed in mmol/L vs heating time and the fitted lines corresponding to the equations given in **Table 4**: (**a**) (*E*)-2-alkenals; (**b**) (*E*,*E*)-2,4-alkadienals; (**c**) *n*-alkanals; (**d**) 4-hydroxy-(*E*)-2-alkenals; (**e**) (*Z*,*E*)-2,4-alkadienals; (**f**) 4-oxo-alkanals.

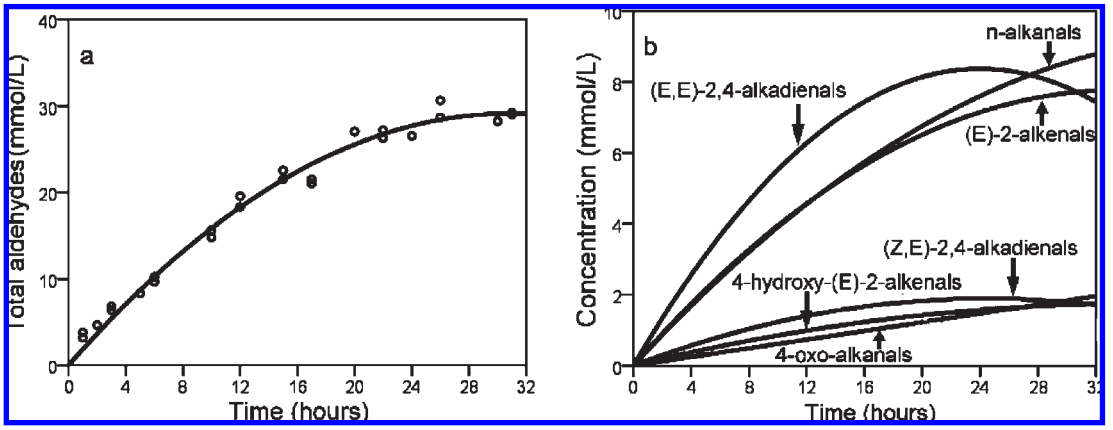

Figure 6. (**a**) Experimental values of the concentration in the sunflower oil of the total aldehydes expressed in mmol/L vs heating time and the fitted line corresponding to the equation in **Table 4**. (**b**) Representation of the equations in **Table 4** obtained by fitting of the concentration of the different kinds of aldehydes in the sunflower oil vs heating time at 190 °C in a domestic fryer.

aldehydes versus heating time follows a parabolic path. The same is true for (*Z*,*E*)-2,4-alkadienals and 4-hydroxy-(*E*)-2-alkenals, being fairly well represented by a linear equation the evolution of 4-oxo-alkanals.

Equations in **Table 4** permit one to predict the concentration of each kind of aldehydes at any heating time. The results thus obtained refer to molar concentrations of aldehydes being supported either on molecules of low molecular weight or on truncated triglycerides or on both. The comparison of the aldehydes concentrations found here with those found in other studies is difficult because the conditions under which the thermal treatment takes place have a great influence and also because there are not many studies of oils submitted to frying temperatures in which the concentration of aldehydes has been determined. Pioneers studies of monitoring by ^{1}H NMR edible oils degradation at frying temperatures have determined the concentration of alkanals and of 2,4-alkadienals in thermally stressed culinary oils after 1 h of heating, finding between 1 and 20 mmol of alkanals/kg of oil, and between 2 and 30 mmol of 2,4-alkadienals/kg of oil (*39*). Obviously the concentration of aldehydes present in the thermally stressed oils is a function of the heating conditions, the amount of oil submitted to the treatment, the air/oil contact surface, and the heating time parameters being of great importance. Under the conditions of this study the concentration of alkanals (0.438 mmol/L of oil) in sunflower oil after 1 h of heating is smaller than the results abovementioned and the same is true for the concentration of alkadienals (0.683 mmol/L of oil for (*E*,*E*)-2,4-alkadienals and 0.154 mmol/L of oil for (*Z*,*E*)-2,4-alkadienals).

In some other previous studies, the concentration of 4-hydroxy-(*E*)-2-nonenal in several soybean oil samples (sample weights varying between 50, 100, 300, and 3 g) submitted at 185−190 °C for different periods of time (3, 5, 6, and 8 h of heating, respectively) under different conditions, was determined. These

Table 5. Heating Time at which the Concentrations of Several Kinds of Aldehydes Reach the Maximum Value, T_{max}, the Predicted Maximum Concentration $[Al]_{max}$, and the Coefficients of the Equations [$dAl/dT = a + bT$] of the Changes in Rate and in the Concentration of Several Kinds of Aldehydes in Relation to the Heating Time

compound group	T_{max} (h)	$[Al]_{max}$ (mmol/L)	equation	*a*	*b*
total aldehydes	30.82	29.166	18	1.8925	−0.0614
(*E*)-2-alkenals	33.67	7.824	19	0.4647	−0.0138
(*E*,*E*)-2,4-alkadienals	23.91	8.344	20	0.6981	−0.0292
n-alkanals	41.83	9.274	21	0.4434	−0.0106
4-hydroxy-(*E*)-2-alkenals	35.53	1.768	22	0.0995	−0.0028
(*Z*,*E*)-2,4-alkadienals	23.87	1.882	23	0.1576	−0.0066
4-oxo-alkanals			24	0.0616	0.0000

determinations were carried out after derivatization, extraction, and successive clean up steps and posterior separation and quantification by high performance liquid chromatography; the concentrations found varied between 0.024, 3.7, 0.27, and 0.016 mmol/kg of oil, respectively (*40*−*43*). The concentrations of all 4-hydroxy-(*E*)-2-alkenals found in this study at the same heating times (3, 5, 6, and 8 h) above-mentioned, deduced from the corresponding equation in **Table 4** are 0.29, 0.46, 0.55, and 0.71 mmol/L of oil; these values are also somewhat different to those found by the authors above and they make reference to the total of 4-hydroxy-(*E*)-2-alkenals supported either on low molecules or on truncated triglycerides.

Recently, other authors (*44*) have determined, after successive steps of extraction and clean up, the concentration of 2,4-decadienal in sunflower oil used for frying in several restaurants in Greece without knowing the degradative conditions to which the oil was submitted. The results obtained range from 0.002 to 0.787 mmol of 2,4-decadienal isomers/kg of oil; in the same study these authors found a value of 0.2 mmol of 2,4-decadienal isomers/kg of oil in sunflower oil after eight successive frying sessions. In the study here carried out the concentrations found, after 4 h of heating at 190 °C (period of time in which approximately eight frying sessions are possible), for both kinds of alkadienal isomers are of 2.56 mmol of (*E*,*E*)-2,4-alkadienals/L of oil and of 0.58 mmol of (*Z*,*E*)-2,4-alkadienals/L of oil; these results deduced from equations in **Table 4** are clearly higher than those reported by the authors above but, as mentioned, this included all (*E*,*E*)-2,4-alkadienals or (*Z*,*E*)-2,4-alkadienals being supported either on small molecules or on truncated triglycerides.

From the equations in **Table 4**, other additional information can also be deduced, such as the heating time at which the concentration of aldehydes reaches the maximum value, the predicted maximum concentration of aldehydes, and also the equations that reflect the rate of change in the concentration of aldehydes in the oil with the heating time [$dAl/dT = a + bT$]. The heating time at which the maximum concentration of total aldehydes (or of each kind of aldehydes) can be reached can be obtained making zero the first derivative of the equation given in **Table 4**, which relates concentration and heating time. **Table 5** gives the heating times at which the maximum concentration is predicted. It can be observed that, in the case of total aldehydes, (*E*,*E*)-2,4-alkadienals and (*Z*,*E*)-2,4-alkadienals, this value is inside the range of time studied; for the remaining aldehydes, their maximum concentration is produced at longer heating times. Likewise, the predicted maximum concentrations in aldehydes can be deduced from equations in **Table 4**; these values are also given in **Table 5**. It can be observed that the maximum concentration predicted is near 30 mmol of total aldehydes/L of oil, near 9 mmol of alkanals/L of oil, near 8 mmol of (*E*)-2-alkenals/L of oil, and near 8 mmol of (*E*,*E*)-2,4-alkadienals/L of oil; the contribution of (*Z*,*E*)-2,4-alkadienals and of 4-hydroxy-(*E*)-2-alkenals are nearly four times smaller. Finally, equations that reflect the rate of change of concentration of the total aldehydes (and of each kind of aldehydes) with the heating time given in **Table 5** indicate that the greatest changes in concentration occur at the beginning of the thermal treatment and that this rate of change diminishes very slowly as the heating time increases. All these results can be observed in a summarized way in **Figure 6b**.

To know the level of degradation of the oil that corresponds with the limit of 25% of the polar compounds, this latter was determined by using a fast test method, based on viscosity measures, namely, the Viscofrit system mentioned before; this test has shown a high correlation with the official method (*9*). As expected, an increase in the viscosity of sunflower oil with heating is produced due to the cross-linking between chains of different triglyceride molecules giving rise to dimers, oligomers, or polymers, and after 26 h of heating, this oil reached the limit of 25% of polar compounds. At this point, the percentage of linoleic acyl groups (L) is near 11% lower than the original due to its degradation, the percentage of monounsaturated acyl groups (MU) is near 2% higher than the original percentage of oleic acyl groups, and the percentage of saturated plus modified groups (S + M) is near 9% higher than the original percentage of saturated groups, which can be attributed to the formation of modified acyl groups derived mainly from linoleic groups degradation and to a much lesser extent from oleic groups degradation because saturated groups hardly degrade. Likewise the iodine value of the oil after 26 h of heating is near 11 units less than that of the original oil; this latter result is in agreement with that of other authors (*4*) who also found a drop of between 5 and 11 units in the iodine values of several oils submitted at 190 °C.

The concentration of total aldehydes when the level of polar compounds in the sunflower is near 25% reaches a value around 28 mmol/L of oil, with the most concentrated being (*E*,*E*)-2,4-alkadienals (8.3 mmol/L of oil), alkanals (8.0 mmol/L of oil), and (*E*)-2-alkenals (7.4 mmol/L of oil) and with (*Z*,*E*)-2,4-alkadienals (1.9 mmol/L of oil), 4-hydroxy-(*E*)-2-alkenals (1.6 mmol/L of oil), and 4-oxo-alkanals (1.6 mmol/L) in much lower concentrations than the first group of aldehydes.

This study was done with oil samples taken from the top and from the bottom of the fryer separately and the results obtained were independent of the sample location, showing that the degradation rate of this oil, under the conditions of this study, is homogeneous in all locations of the fryer tank, as it is the formation of aldehydes; this can be expected because the oil liquid phase is in continuous movement during heating.

In summary, heating sunflower oil at 190 °C in a domestic fryer provokes its degradation from the beginning of heating. This degradation mainly affects to linoleic acyl groups, which produces the formation of dimers, trimers, oligomers, and polymers probably by carbon−carbon linkages increasing the viscosity of the oil. The formation of carbon−carbon linkages between linoleic groups could explain the increase in the proportion of monounsaturated acyl groups in the oil in spite of the possibility that a certain proportion of oleic groups can also be degraded in the process by formation of carbon−carbon linkages or by other

reactions. The degradation of linoleic or of oleic acyl groups can also give rise to the formation of molecules of low molecular weight and of truncated triglycerides. The evolution throughout the heating time of the proportions of linoleic, monounsaturated, and saturated plus modified acyl groups in the oil, as well as the iodine value, can be determined from the ^{1}H NMR spectral data, and equations that relate these parameters and the heating time with very high correlation coefficients were found; these equations provide a great deal of information about the evolution of sunflower oil submitted to 190 °C in a domestic fryer. The presence in the sunflower oil of primary oxidation compounds having hydroperoxy and *cis*,*trans* or *trans*,*trans* conjugated dienic systems was not detected; by contrast, the presence of several kinds of aldehydes, some of which have dienic conjugated systems, was detected very early and their concentrations were determined from ^{1}H NMR spectral data. It is evident that some kinds of aldehydes such as alkanals, (*E*)-2-alkenals, and (*E*,*E*)-2,4-alkadienals are formed in much higher proportions than 4-hydroxy-(*E*)-2-alkenals, (*Z*,*E*)-2,4-alkadienals, and 4-oxo-alkanals. Equations that relate, with high correlation coefficients, concentrations of these compounds and heating time were also found. These equations contain a great deal of information about the evolution of these so-called secondary oxidation compounds throughout the heating process at frying temperature during prolonged periods of time. The most significant features when the oil reaches the limit of 25% of polar compounds (determined by viscosity measures) is that the proportion of linoleic acyl groups has diminished near 11%, that of monounsaturated acyl groups and that of saturated plus modified acyl groups have increased near 2 and 9%, respectively, and the iodine value has decreased near 11 units; furthermore, the concentration of total aldehydes, and of the different kinds of aldehydes, is very close to its maximum value. To the best of our knowledge, this is the first time that the proportions of the several acyl groups and the concentrations of the different types of aldehydes have been determined in sunflower oil submitted to frying temperatures and also when the limit of 25% of polar compounds is reached. Taking into account that aldehydes are present in significant concentrations before this limit is reached, being present among them genotoxic and cytotoxic aldehydes, studies directed toward the establishment of safe limits in function of aldehyde content should be considered.

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