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Monitoring of minor compounds in corn oil oxidation by direct immersion-solid phase microextraction-gas chromatography/mass spectrometry. New oil oxidation markers

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6,10,14-Trimethylpentadecan-2-one (PubChem CID: 10408)
α-Tocopherylquinone-5,6-epoxide (PubChem CID: 14753697)
3,7,11-Trimethyl-3-dodecanol (PubChem CID: 138824)
4,8,12,16-Tetramethylheptadecan-4-olide (PubChem CID: 567149)

ABSTRACT

The aim of this study is to shed light on the evolution of the minor compounds in the corn oil oxidation process, through the information provided by direct immersion-microextraction in solid phase followed by gas chromatography/mass spectrometry (DI-SPME-GC/MS). This methodology enables one, in a single run, to establish the identity and abundance both of original oil minor components, some with antioxidant capacity, and of other compounds coming from both main and minor oil components oxidation. For the first time, some of the compounds formed from oil minor components degradation are proposed as new markers of oil incipient oxidation. Although the study refers to corn oil, the methodology can be applied to any other edible oil and constitutes a new approach to characterizing the oxidation state of edible oils.

1. Introduction

Vegetable oils are well known food ingredients and cooking media all over the world. They undergo great changes in their composition under oxidative conditions. These are due to the degradation of some of their components and to the formation of other new compounds. As a consequence, the safety, nutritional value and sensory characteristics of the oil may be affected.

For these reasons, it is of great importance to know the evolution of the original oil components and the identity and characteristics of the new formed compounds, when the oil is submitted to oxidative conditions.

The most common methods used to evaluate the oxidation degree of edible oils require chemical modification of the sample, and provide limited information about the identity of compounds whose functional groups are measured. For example, peroxide or conjugated diene values measure the abundance of both kinds of functional groups; however, there are both primary and secondary oxidation compounds that can have hydroperoxide groups and the same can be said of the conjugated diene group, as has been widely discussed (Frankel, 2005). These approaches are valid, to some extent, for certain objectives but provide no useful information to further knowledge of the different types of reactions which occur, simultaneously or successively, in the process, or about their mechanisms. Nor do they give specific information

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concerning the nature of the different kind of compounds that may form during oil oxidation.

Another approach with the same aim is based on the use of ^{1}H NMR spectroscopy. This technique permits the qualitative and quantitative characterization of main edible oil components (Guillén & Ruiz, 2003; Guillén & Uriarte, 2012a; Martínez-Yusta, Goicoechea, & Guillén, 2014) and it is very useful in monitoring their oxidation because it allows one to evaluate the rate of degradation of the oil main components as well as the formation rate of primary and of many secondary oxidation products (Guillén & Ruiz, 2004; Guillén & Uriarte, 2012a; Martínez-Yusta et al., 2014).

In this context, this paper addresses the characterization of the oxidative status of edible oils, specifically of corn oil, as well as their evolution under oxidative conditions with a new approach based exclusively on the minor compounds contained in the oil. To this end, a fairly new methodology that employs direct immersion solid-phase microextraction to extract oil minor compounds up to a certain molecular weight, followed by gas chromatography/mass spectrometry (Alberdi-Cedeño, Ibargoitia, Cristillo, Sopelana, & Guillén, 2017) will be used. Both approach and methodology have not been used previously in edible oil oxidation studies. They permit the simultaneous monitoring of the original minor oil components and of other compounds coming from the oxidation of main and minor oil components, formed under accelerated storage conditions. Furthermore, this methodology does not require chemical modification of the sample, nor the use of solvents, reagents or fractionation steps.

2. Materials and methods

2.1. Samples subject of study

2.1.1. The study was carried out with refined corn oil, purchased in a local supermarket. Its composition in acyl groups was determined from ^{1}H NMR spectral data as in previous studies (Guillén & Ruiz, 2003, Guillén & Uriarte, 2012a). The molar percentages of the different kinds of acyl groups were linolenic group (Ln) 0.6 ± 0.0%, linoleic group (L) 48.7 ± 0.0%, oleic group (O) 33.0 ± 0.1%, and saturated groups (S) 17.7 ± 0.1%. The peroxide value of this oil was < 0.5 meq O_2/Kg. It was determined by an adaptation of the official method described in Commission regulation EC2568/91, by using the HI 83730 equipment from Hanna Instruments (range: 0.0–25 meq O_2/Kg; resolution: 0.5 meq O_2/Kg; accuracy: ± 0.5 meq O_2/Kg). The volatile and less volatile minor components of this oil were extracted by means of direct immersion-solid phase microextraction and were separated, identified and semi-quantified by gas chromatography/mass spectrometry as indicated in Point 2.2.

2.1.2. In addition to the original oil, samples also derived from this oil, after subjected to accelerated storage conditions for different periods of time, were subject of study. To prepare these derived samples amounts of 10 g of original corn oil were placed in glass Petri dishes (80 mm in diameter and 15 mm deep) and kept in an oven at 70 °C with aeration for three, six, nine and twelve days. Under these conditions the original oil underwent oxidation. These experiments were performed in duplicate to obtain sound results. The volatile and less volatile minor compounds of these oxidized oil samples were extracted by means of direct immersion-solid phase microextraction and were separated, identified and semi-quantified by gas chromatography/mass spectrometry, as indicated in Point 2.2.

2.1.3. Pure squalene, α- and γ-tocopherol were subjected to the same oxidative conditions as above and were studied by GC/MS. To this aim, aliquots of these oxidized samples were conveniently dissolved in dichloromethane and injected directly in the chromatograph. The chromatographic conditions were the same as described in Point 2.2.2. In this way the origin of some new compounds found in the oxidized corn oil samples could be confirmed.

2.2. Direct immersion-solid phase microextraction followed by gas chromatography/mass spectrometry (DI-SPME-GC/MS) experiments

2.2.1. Extraction of the minor compounds contained in the samples subject of study

The minor compounds contained either in the original oil or in its derived samples were extracted by direct immersion-solid phase microextraction without any previous sample preparation. To this aim a 65 µm StableFlex polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber acquired from Supelco (Bellefonte, PA, U.S.) was immersed directly into 5.4 g of edible oil, at room temperature, for 45 min, maintained under continuous stirring. The selection of the type of fiber (polarity and thickness of the coating) was made on the basis of its ability to extract the widest range of minor oil components with the highest possible yield, after analyzing the results obtained by Mikuma and Kaneko (2010) and after testing several polymer-coated fused silica fibers, as in a previous study (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017).

2.2.2. Gas chromatography/mass spectrometry study (GC/MS)

The oil components picked up by the fiber were directly injected into the port of a gas chromatograph/mass spectrometer (GC/MS). For their thermal desorption, the plunger was pushed down to expose the fiber to the GC carrier gas stream and held for 10 min. The desorbed compounds were separated, identified and semi-quantified by gas chromatography-mass spectrometry.

The equipment used was an Agilent gas chromatograph model 6890N equipped with a mass selective detector 5973 Network and a Hewlett-Packard Compaq Pentium 4 computer. A fused silica capillary column was used (60 m length × 0.25 mm inside diameter × 0.25 µm film thickness; from Hewlett-Packard, Palo Alto, CA), coated with a nonpolar stationary phase (HP-5MS, 5% phenyl methyl siloxane). The injector and interface temperatures were held at 250 °C and 305 °C respectively and helium at a constant pressure of 117 kPa (16.9 psi) was used as the carrier gas. The oven temperature was initially held at 50 °C for 5 min, increased from 50 to 300 °C at a rate of 4 °C/min, and then held at 300 °C for 30 min. The injections were carried maintaining the fiber in the injection port for 10 min, the first 5 in splitless mode. Mass spectra were recorded at ionisation energy of 70 eV, with data acquisition in Scan mode. The Scan range was 40–550 amu. The temperatures of the ion source and the quadrupole mass analyzer were kept at 230 and 150 °C respectively. After the first desorption, the fibre was routinely submitted to desorption conditions for a second time both for its clean up and to determine if the first process had been completed. A reference sample of known composition was periodically analyzed in order to verify the reproducibility and sensitivity of the chromatographic runs.

The identification of the several compounds was made using standards in some cases and on the basis of their mass spectra and retention times in others. The standards used, asterisked in Tables 1, 2, S1, S2 and S3, were acquired from Sigma Aldrich (St. Louis, MO, USA), Bachem AG and Carbosynth Ltd (Cymit Quimica S.L, Barcelona, Spain), Larodan (Malmö, Sweden), Cayman Chemical (Ann Arbor, MI, USA), Toronto Research Chemicals, (Canada; Commercial Rafer, Spain), LGC Standards, (Barcelona, Spain) and ChemFaces Biochemical Co., LTD (Wuhan, China). The identification of the others was made by matching their mass spectra with spectra from commercial libraries by more than 85% (W9N08, Wiley ver. 9.0 & NIST, ver. 8.0 library) with spectra obtained from the literature (Cornwell, Kim, Mazzer, Jones, & Hatcher, 2003; Nassiry, Aubert, Mouzdahir, & Rontani, 2009; Rontani, Nassiry, Michotey, Guasco, & Bonin, 2010; Rontani, Nassiry, & Mouzdahir, 2007) and taking also into account their retention times as in previous studies (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017; Alberdi-Cedeño, Ibargoitia, & Guillén, 2017; Goicoechea & Guillén, 2014; Guillén, Cabo, Ibargoitia, & Ruiz, 2005). This identification method is accepted for food studies where, as in this case, their composition is

Table 1
Evolution of the abundances of tocols, sterols, squalene and DKPs present in corn oil submitted to oxidative conditions during different periods of time, expressed as area counts of their mass spectra base peak (Bp) multiplied by 10^{-5}, obtained as average of two determinations together with their standard deviation.

Compound (molecular weight)	Bp	Time (days)				
		0	3	6	9	12
Tocols						
Tocopherols						
δ-tocopherol (402)*	402	41.5 ± 0.2	40.1 ± 0.0	23.7 ± 2.4	5.6 ± 1.0	0.6 ± 0.1
β-tocopherol (416)*	416	11.1 ± 0.1	11.0 ± 0.3	6.6 ± 0.6	–	–
γ-tocopherol (416)*	416	1,135.1 ± 54.4	1,064.7 ± 21.9	459.9 ± 12.5	16.5 ± 1.6	4.0 ± 0.5
α-tocopherol (430)*	165	220.1 ± 13.6	200.9 ± 6.3	40.5 ± 9.9	3.9 ± 0.0	2.6 ± 0.4
Tocomonoenols						
γ-tocomonoenol (414)	414	4.3 ± 0.1	3.8 ± 0.1	1.3 ± 0.1	–	–
α-tocomonoenol (428)	428	4.0 ± 0.2	3.4 ± 0.2	0.5 ± 0.1	–	–
Tocotrienols						
γ-tocotrienol (410)*	151	7.2 ± 0.3	6.7 ± 0.5	2.6 ± 0.3	–	–
α-tocotrienol (424)*	165	11.0 ± 1.0	9.7 ± 0.6	3.1 ± 0.1	–	–
Sterols-stanol						
Campesterol (400)*	400	14.3 ± 0.1	13.9 ± 0.6	9.1 ± 0.9	6.2 ± 0.1	2.7 ± 0.0
Stigmasterol (412)*	412	2.8 ± 0.1	2.6 ± 0.3	1.6 ± 0.3	1.2 ± 0.0	0.4 ± 0.1
β-sitosterol (414)*	414	32.7 ± 0.5	31.3 ± 1.9	22.2 ± 2.9	14.2 ± 0.4	6.5 ± 0.5
Sitostanol (416)*	215	1.1 ± 0.1	1.0 ± 0.0	0.8 ± 0.0	0.5 ± 0.0	–
Δ5-avenasterol (412)*	314	2.5 ± 0.0	2.4 ± 0.2	1.4 ± 0.1	1.3 ± 0.0	–
Terpenic hydrocarbon						
Squalene (410)*	69	539.8 ± 24.0	476.8 ± 1.5	461.3 ± 22.7	433.9 ± 28.4	117.5 ± 22.0
DKPs						
Cyclo (Phe-Val)I (246)*	91	0.8 ± 0.1	0.6 ± 0.1	0.5 ± 0.0	–	–
Cyclo (Phe-Val)II (246)	91	0.9 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	–	–
Cyclo (Leu-Phe)I (260)*	91	0.7 ± 0.1	0.6 ± 0.0	0.6 ± 0.0	–	–
Cyclo (Leu/Ile-Phe)II (260)	91	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	–	–
Cyclo (Pro-Phe)I (244)*	125	0.6 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	–	–
Cyclo (Leu/Ile-Phe)III (260)	91	1.2 ± 0.2	1.0 ± 0.0	0.9 ± 0.1	–	–
Cyclo (Leu/Ile-Phe)IV (260)	91	0.8 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	–	–
Cyclo (Pro-Phe)II (244)	125	1.3 ± 0.0	1.2 ± 0.1	0.9 ± 0.0	0.6 ± 0.1	–

* Asterisked compounds were acquired commercially and used as standards for identification purposes; –: no detected.

very complex, the number of components is very high, the components subject of study can be present in very low concentration, the molecules are not new, and their mass spectra are well known and established (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017; Alberdi-Cedeño, Ibargoitia, & Guillén, 2017; Goicoechea & Guillén, 2014; Guillén et al., 2005).

The semi-quantification of the identified compounds was based on arbitrary units of area counts, of the corresponding mass spectra base

Table 2
Abundances of the compounds formed by the oxidation of minor corn oil components at different periods of time under oxidative conditions, expressed as area counts of their mass spectra base peak (Bp) multiplied by 10^{-5}, obtained as average of two determinations together with their standard deviations.

Compound (molecular weight)	Bp	Time (days)				
		0	3	6	9	12
Tocopherol oxidation derived compounds						
Prist-1-ene (266) (P)	56	1.0 ± 0.1	1.7 ± 0.1	4.1 ± 0.4	10.2 ± 0.5	10.6 ± 1.6
6,10,14-trimethylpentadecan-2-one (268) (TrMPD)	43	0.6 ± 0.0	1.4 ± 0.0	1.6 ± 0.2	5.0 ± 0.5	15.2 ± 3.8
3,7,11-trimethyl-3-dodecanol (228) (TrMD)	73	–	–	1.6 ± 0.0	3.0 ± 0.2	10.9 ± 0.2
4,8,12,16-tetramethylheptadecan-4-olide (324) (TeMHD)	99	–	2.2 ± 0.2	13.2 ± 1.7	35.5 ± 3.1	105.3 ± 25.8
α-tocopherylquinone-2,3-epoxide (462) (TQ23E)	237	–	–	1.9 ± 0.6	2.9 ± 0.3	2.2 ± 0.6
α-tocopherylquinone-5,6-epoxide (462) (TQ56E)	237	–	–	1.1 ± 0.2	1.6 ± 0.1	–
β-sitosterol oxidation derived compounds						
7-ketositosterol (428)*	428	–	–	–	–	0.8 ± 0.1
Squalene oxidation derived compounds						
Farnesal (220)*	69	–	–	–	1.4 ± 0.2	28.8 ± 9.4
Farnesal (isomer) (220)	69	–	–	–	–	4.4 ± 0.3
Farnesyl acetate (264)	69	–	–	–	3.3 ± 0.9	13.3 ± 4.8
2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene (342)	69	–	–	–	–	4.1 ± 1.5
Farnesol (isomer) (222)	69	–	–	–	–	4.1 ± 1.1
10,11-epoxysqualene (426)	69	–	–	–	5.6 ± 0.6	13.2 ± 1.5
2,3-epoxysqualene (426)*	69	–	–	–	9.1 ± 0.0	17.5 ± 1.7
Squalen-22-one (426)	69	–	–	–	5.6 ± 0.9	13.1 ± 1.5
2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaenal (424)	69	–	–	–	–	7.9 ± 0.2

* Asterisked compounds were acquired commercially and used as standards for identification purposes; –: no detected.

peaks, multiplied by 10^{-5}. The purpose of this study was not the determination of absolute but rather of relative concentrations that are valid for comparative purposes.

2.3. Quality control of the global method and statistical analysis

Quality control of the global method was carried out by periodic extraction of an oil sample considered as sample reference and the subsequent study of the extracted compounds by GC/MS. The detection limit was established in an abundance of 10,000 area counts for the base peak of the mass spectra of the compounds. The specificity, selectivity and robustness of the GC/MS to evaluate unequivocally the compounds included in this study are beyond all doubt as it is a recognized suitable global method for the purposes of this study. Data given in Tables 1, 2 and Tables S1, S2 and S3 are expressed as mean ± standard deviation (SD) of two measurements for the analytical determination. Microsoft Office Excel 2007 was used for the graphical representation of the obtained values. The global results here obtained have been corroborated with three other different corn oils submitted to the same oxidative conditions.

3. Results and discussion

3.1. Minor compounds present in the original corn oil

As mentioned above, characterization of the original corn oil minor components was the starting point of this study. It provided information about the identity and abundance of a broad range of compounds, some of which can have antioxidant ability, others which come from oil oxidation and provide information about its oxidation status, and others which are of varied origin and nature.

3.1.1. Compounds with antioxidant ability

These include squalene, tocols, sterols, and some cyclic dipeptides or DKPs (2,5-diketopiperazines) (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017; Alberdi-Cedeño, Ibargoitia, & Guillén, 2017). Most of these, except DKPs, eluted in the chromatographic run after sixty-five minutes, as can be seen in Fig. 1.

The main *tocol* is γ-tocopherol (γ-T; peak 27 in Fig. 1) followed by α - and δ-tocopherols (α-T and δ-T; peaks 28 and 25 respectively, in Fig. 1) and in much smaller abundances β-tocopherol (β-T; peak 26 in Fig. 1), as well as some tocomonoenols and tocotrienols. All of them elute in the chromatographic run between sixty-nine and seventy five minutes (Fig. 1).

Like most edible oils, corn oil contains several *sterols*, mainly β-sitosterol (β-S; peak 30 in Fig. 1), followed by campesterol (C; peak 29 in Fig. 1) and in much smaller abundance stigmasterol (STG), Δ5-avenasterol (Δ5-A) and one stanol, sitostanol (STN) (see Table 1). All these compounds elute after seventy six minutes (Fig. 1).

The abundances, within each one of these two groups of compounds above mentioned, measured in area counts, follow a fairly similar relative order to those of their absolute concentrations (Ostlund, Racette, Okeke, & Stenson, 2002; Phillips, Ruggio, Toivo, Swank, & Simpkins, 2002; Shahidi & de Camargo, 2016). This is the case within each kind of compound because of the similarity in functional groups and structure and also due to the proximity of their retention times. However, as commented above, and as is well known, the abundance values thus determined are only valid to compare the evolution of the same compound in several samples.

In addition to the above-mentioned corn oil components, this oil also contains *cyclic dipeptides or DKPs*. To the best of our knowledge, the only edible oil in which the presence of this type of compounds has been described to date is corn oil (Alberdi-Cedeño, Ibargoitia, & Guillén, 2017). They are, as usual, in very small abundance. The DKPs found in this corn oil derive from phenylalanine (Phe) and other amino acids such as valine (Val) (isomers Cyclo(Phe-Val)I and Cyclo(Phe-Val) II, in Table 1), leucine (Leu), isoleucine (Ile) (isomers Cyclo(Leu-Phe)I, Cyclo(Leu/Ile-Phe)II, Cyclo(Leu/Ile-Phe)III and Cyclo(Leu/Ile-Phe)IV, in Table 1) and proline (Pro) (isomers Cyclo(Pro-Phe)I and Cyclo(Pro-Phe)II, in Table 1). Those found here are among the most abundant found in other corn oils (Alberdi-Cedeño, Ibargoitia, & Guillén, 2017). They elute between 56 and 60 min in the chromatographic run and due to their low concentration their chromatographic peaks are not clearly visible with the naked eye, for which reason they are not indicated in Fig. 1.

3.1.2. Compounds coming from oil oxidation

This oil also contains a basal concentration of some compounds whose origin could be in the oxidation of main corn oil components. These elute in the first thirty-three minutes of the chromatographic run, as can be seen in Fig. 1 day 0. Among them there are aldehydes, of which the most abundant are some *alkanals* such as pentanal, hexanal (peak 1 in Fig. 1) and nonanal (peak 11 in Fig. 1), some *(E)-2-alkenals* such as (*E*)-2-heptenal (peak 5 in Fig. 1), (*E*)-2-octenal (peak 10 in Fig. 1), (*E*)-2-decenal (peak 16 in Fig. 1), and (*E*)-2-undecenal (peak 21 in Fig. 1), some *2,4-alkadienals* such as (*Z,E*)- and (*E,E*)-2,4-decadienal (peaks 17 and 18 respectively, in Fig. 1) and some *oxygenated alpha,beta unsaturated aldehydes* like, 4-hydroxy-(*E*)-2-nonenal (peak 20 in Fig. 1) and 4,5-epoxy-(*E*)-2-decenal (peak 23 in Fig. 1) (see Table S1). Likewise, small abundances of *2-pentylfuran* (peak 7 in Fig. 1), a reduced number of *alcohols*, *ketones*, some *furanones* and *lactones* were also found (see Table S2 and Fig. 1).

3.1.3. Other minor compounds

Finally, a third group of compounds was found in this corn oil (see Table S3). They are a reduced number of *glycidyl fatty acid esters* (GEs). They are considered toxic contaminants (Hoogenboom, 2016), supposedly formed in the refining process (Cheng, Liu, & Liu, 2016). The abundance of these compounds is, as expected, very low.

3.2. Evolution under oxidative conditions of the antioxidant compounds present initially in the corn oil

When oil is subjected to oxidative conditions, it is to be expected that the antioxidants, initially present, undergo degradation, thus decreasing in concentration. The rate of this degradation will depends on the oxidative stability of each one of these compounds under these conditions.

3.2.1. Degradation kinetics of tocols

The abundances of the various *tocopherols* in the oil, at different days under oxidative conditions, are given in Table 1 and represented in Fig. S1A. It can be observed that they remained practically constant during an initial period of time (approximately up to day 3 under degradative conditions). However, from day 3 until day 9, they underwent a rapid degradation. The abundance of each one of these tocopherols **[T]** and time, **t**, under degradative conditions (considering day 3 the starting point (time = 0) and day 9 the end point (time = 6)) fitted, with a high correlation coefficient, to the general equation $\mathbf{[T] = m\,t + b}$, being **m** and **b** specific of each tocopherol. The equations obtained were: $\mathbf{[\gamma\text{-}T] = -174.7\ t + 1037.8}$ ($R = 0.9961$, $n = 3$); $\mathbf{[\alpha\text{-}T] = -32.8\ t + 180.3}$ ($R = 0.9401$, $n = 3$); $\mathbf{[\delta\text{-}T] = -5.7\ t + 40.4}$ ($R = 0.9960$, $n = 3$); and $\mathbf{[\beta\text{-}T] = -1.8\ t + 11.4}$ ($R = 0.9934$, $n = 3$). In these equations the abundance **[T]** is given in area counts of the base peak of the mass spectra of the corresponding tocol multiplied by 10^{-5} and **t** is given in days. The degradation rate **m** of each tocopherol, derived from these equations, is defined by $\mathbf{m = d[T]/dt}$. The highest degradation rate was shown by γ-T, followed by α-T, δ-T and β-T. Furthermore, it was also observed that there is a very close relationship between the degradation rate **m** of each tocopherol thus obtained and its abundance at time 0. In fact, both latter parameters, including the four tocopherols, are linearly related with a correlation coefficient of 1.

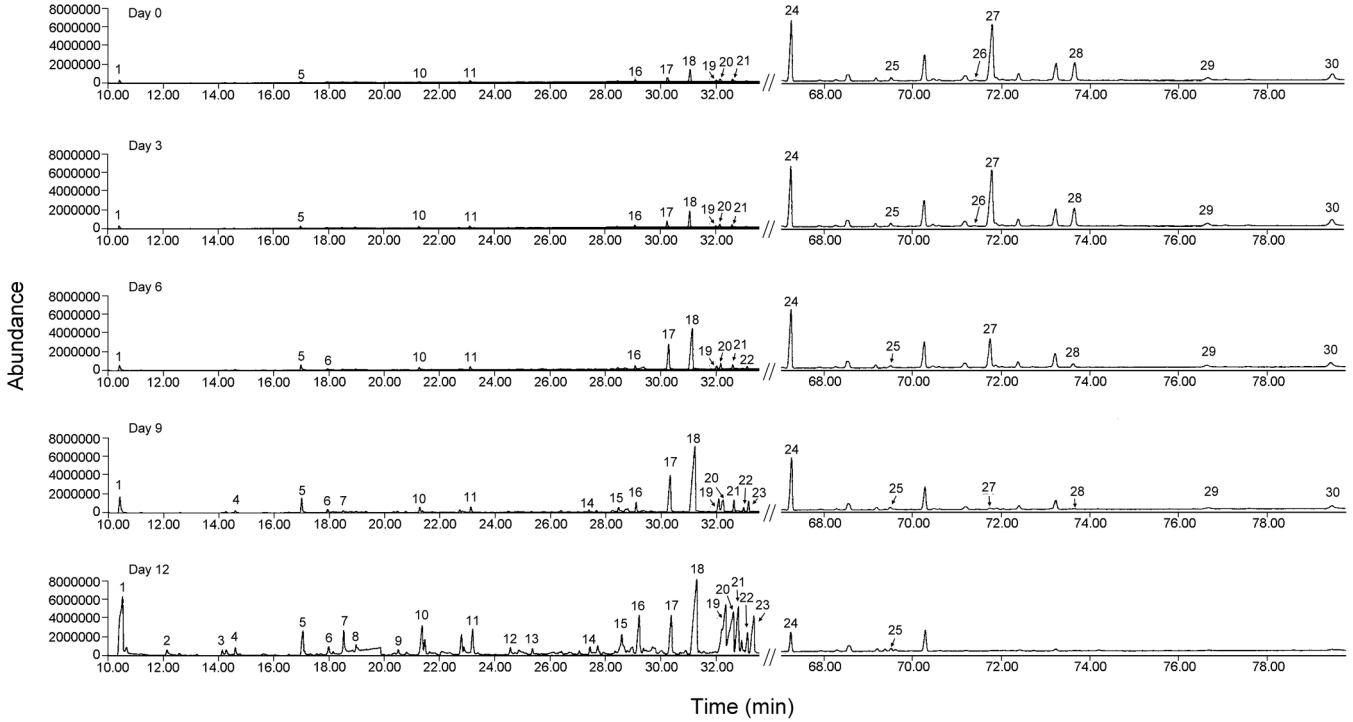

Fig. 1. Regions between 10–32 min and 67–80 min of the total ion chromatograms obtained by DI-SPME-GC/MS of corn oil submitted during different periods of times to the same oxidative conditions. Peaks identified as: **(1)** hexanal; **(2)** (*E*)-2-hexenal; **(3)** 2-heptanone; **(4)** heptanal; **(5)** (*E*)-2-heptenal; **(6)** 1-octen-3-ol; **(7)** 2-pentylfuran; **(8)** octanal; **(9)** 3-octen-2-one; **(10)** (*E*)-2-octenal; **(11)** nonanal; **(12)** 3-nonen-2-one; **(13)** (*E*)-2-nonenal; **(14)** (*E,E*)-2,4-nonadienal; **(15)** 4-oxo-(*E*)-2-nonenal; **(16)** (*E*)-2-decenal; **(17)** (*Z,E*)-2,4-decadienal; **(18)** (*E,E*)-2,4-decadienal; **(19)** 5-pentyl-2-(5H)-furanone; **(20)** 4-hydroxy-(*E*)-2-nonenal; **(21)** (*E*)-2-undecenal; **(22)** 4,5-epoxy-2-decenal (isomer); **(23)** 4,5-epoxy-(*E*)-2-decenal; **(24)** squalene; **(25)** δ-tocopherol; **(26)** β-tocopherol; **(27)** γ-tocopherol; **(28)** α-tocopherol; **(29)** campesterol and **(30)** β-sitosterol.

In summary, it can be said that the degradation of these four tocopherols contained in corn oil fits well with a kinetic model of zero-order, under the oxidative conditions of this study.

Likewise, a first-order kinetic model $Ln[\mathbf{T}] = Ln[\mathbf{T}]_0 + \mathbf{k}\ \mathbf{t}$ was tested. In this model **T** and **t** meaning is the same as above, **k** being the degradation rate constant. The equations obtained ($Ln[\gamma\text{-}\mathbf{T}] = 7.4–0.7\ \mathbf{t}$ ($R = 0.9454$, $n = 3$); $Ln[\boldsymbol{\alpha}\text{-}\mathbf{T}] = 5.4–0.7\ \mathbf{t}$ ($R = 0.9942$, $n = 3$); $Ln[\boldsymbol{\delta}\text{-}\mathbf{T}] = 3.8–0.3\ \mathbf{t}$ ($R = 0.9657$, $n = 3$); $Ln[\boldsymbol{\beta}\text{-}\mathbf{T}] = 2.4–0.2\ \mathbf{t}$ ($R = 1.0000$, $n = 2$) also fit fairly well, however the correlation coefficients were somewhat worse than those obtained with the zero-order kinetic model. With this new approach the degradation rate of each tocopherol at time **t**, $(\mathbf{r})_\mathbf{t}$, is a function of its abundance **T**, at this same time, $[\mathbf{T}]_\mathbf{t}$, as indicated in the equation $(\mathbf{r})_\mathbf{t} = \mathbf{k}\ [\mathbf{T}]_\mathbf{t}$, where **k** has the same meaning as above. In this approach γ-T, also shows the highest degradation rate followed by α-T, δ-T and β-T.

A comparison of the results here obtained with those obtained by other authors is not easy because the systems involved, degradative conditions, analytical tools employed and data treatment are usually different.

Regarding studies in model systems, the degradation of α-T in methyl linoleate, during storage, fitted well to a zero-order kinetic model (Widicus, Kirk, & Gregory, 1980) like here. However, a first-order kinetic model was considered the best to describe the degradation of α-, γ- and δ-T separately in glycerol (Chung, 2007); in this latter study δ-T and γ-T showed higher degradation rate than α-T.

With respect to the studies of edible oils, it can be mentioned that it refers to the degradation of the same three tocopherols cited above in soybean oil stored in bottles sealed at 50 °C. (Player, Kim, Lee, & Min, 2006). Although no kinetic study was made, the results indicated that α-T disappeared from the oil before γ -and δ-T. Nevertheless, it should be mentioned that in soybean oil the concentration of γ-T and δ-T, is much higher than that of α-T, and this could influence on its early disappearance (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017; Cerretani, Lerma-García, Herrero-Martínez, Gallina-Toschi, & Simó-Alfonso, 2010). Other authors studied the rate of degradation of the same three tocopherols above mentioned contained in perilla and corn oils maintained at different temperatures (20, 40, 60 and 80 °C) in bottles closed with Korean paper (Wang, Hwang, Yoon, & Choe, 2010). No clear effect of temperature on the degradation rate of the same tocopherol in the same oil was found, and the order in the degradation rate of the three tocopherols was not the same in the same oil at different temperatures.

The evolution of *tocomonoenol* and *tocotrienol* abundances followed a similar path as tocopherols, under the oxidative conditions of this study, and after 9 days all of them disappear from the corn oil, as Table 1 shows.

3.2.2. Degradation kinetics of sterols

The abundance of these compounds remained, like that of tocols, practically unchanged until day 3 (see Table 1 and Fig. S1B). Abundance and time, from day 3 ($\mathbf{t} = 0$) to day 12 ($\mathbf{t} = 9$) under oxidative conditions, fitted well to linear equations. The equations found for the main sterols, campesterol (**C**), and β-sitosterol (**β-S**), were $[\mathbf{C}] = -1.2\ \mathbf{t} + 13.5$ ($R = 0.9945$, $n = 4$) and $[\boldsymbol{\beta}\text{-}\mathbf{S}] = -2.8\ \mathbf{t} + 30.9$ ($R = 0.9992$, $n = 4$). These high correlation coefficients indicate that also in this case the degradation of sterols fits well to a zero-order kinetic model, the degradation rate of β-sitosterol being higher than that of campesterol. The equations obtained considering a first-order kinetic model also have high correlation coefficients although somewhat smaller than those of the zero-order kinetic model ($Ln[\mathbf{C}] = -0.2\ \mathbf{t} + 2.7$ ($R = 0.9814$, $n = 4$) and $Ln[\boldsymbol{\beta}\text{-}\mathbf{S}] = -0.2\ \mathbf{t} + 3.5$ ($R = 0.9815$, $n = 4$)); in this second model the degradation rate of β-sitosterol is also higher than that of campesterol. As in the case of tocols, the degradation rate of sterols is strongly affected by the oxidative conditions. No

degradation of sterols in oils has been observed under very mild oxidation conditions (low temperature, dark and absence of oxygen), whereas under stronger oxidative conditions a relevant degradation level has been detected (Gawrysiak-Witulska, Rudzińska, Siger, & Bartkowiak-Broda, 2015; Oehrl, Hansen, Rohrer, Fenner, & Boyd, 2001; Thanh et al., 2006). However, to the best of our knowledge, kinetic studies on sterols degradation in vegetable oils have not been carried out.

3.2.3. Degradation kinetics of squalene

Another important minor corn oil component is squalene, Sq. As Table 1 and Fig. S1C show, the abundance of this compound decreases very slowly from day 0 to day 9 and then very sharply from day 9 to day 12. In the first stage (days 0–9), the abundance [**Sq**] and time **t** fit, with a high correlation coefficient, to the linear equation [**Sq**] = −11.1 **t** + 527.9 (R = 0.9583, n = 4) and also to the equation Ln [**Sq**] = −0.02 **t** + 6.3 (R = 0.9660, n = 4). This indicates that squalene degradation is fairly well described by both either a zero-order or a first-order kinetic model. In a previous study, of olive oil subjected to storage conditions, it was proposed that α-tocopherol exerts a protective effect on squalene by avoiding or retarding its degradation (Rastrelli, Passi, Ippolito, Vacca, & De Simone, 2002). This could explain the slow degradation rate of this compound found here from day 0 to day 9, although this could also be due to the oxidative stability of squalene exclusively.

3.2.4. Evolution of DKPs

As mentioned before, this oil, like other corn oils, also contains *DKPs* in very small abundance. Table 1 shows the evolution of their abundances over time under oxidative conditions. The degradation of these compounds appears to begin from day 0, and on day 9 they are totally degraded. Due to their low abundance the equations relating their abundance and time under degradative conditions are more affected by experimental errors than those of the components in higher abundance mentioned above, for which reason no kinetic data are given.

In summary, it is evident that under oxidative conditions, from day 3 to day 9, the abundance of tocols has been reduced nearly ten times, whereas that of sterols has been reduced about a half and that of squalene by a factor of around 0.8. From day 9 onwards the degradation rate of all of these oil components increases considerably and on day 12 the abundance of many of them is small and that of others null.

3.3. Formation of compounds derived from the antioxidant components of corn oil and evolution of their abundance

The degradation of each one of the above-mentioned minor corn oil components can lead to the formation of several derived compounds which were not present in the original oil or that were present in very low abundance. As the parent compounds are minor oil components, the abundance of their derived compounds will be very small. For this reason, their detection is not easy. This may be the reason why, in most of the studies dedicated to analyzing the evolution, under oxidative conditions, of oil antioxidant components, their derived compounds were not contemplated (Player et al., 2006; Thanh et al., 2006; Wang et al., 2010). Nevertheless, the methodology here used allows one to identify and determine the abundance of some of these derived compounds.

3.3.1. Compounds coming from tocopherols degradation

Compounds found coming from tocopherol degradation can be placed in two groups: one constituted by compounds derived from any of the four tocopherols and a second group constituted by those derived specifically from α-T.

The first group comprises prist-1-ene (P), 6,10,14-trimethylpentadecan-2-one (TrMPD), 3,7,11-trimethyl-3-dodecanol (TrMD) and 4,8,12,16-tetramethylheptadecan-4-olide (TeMHD) (see Fig. 2A and Table 2). The first two compounds (P and TrMPD) were present, in very low abundance, in the original corn oil, due to the basal oxidation level present in edible oils. Some authors have reported that these two compounds can be formed from tocopherols under very different degradative conditions (Goossens, Leeuw, Schenck, & Brassell, 1984; Nassiry et al., 2009). The formation of TrMD and TeMHD as coming from tocopherol degradation has been proved in our laboratory, in parallel studies of pure tocopherol oxidation submitted to 70 °C with aeration. To the best of our knowledge, this is the first time that the formation of these two latter compounds has been described in edible oil oxidation.

The abundance of these tocopherol derivatives increases with time under oxidative conditions (see Table 2). As mentioned, two of them, P and TrMPD, were present in the original oil, and TeMHD was detected

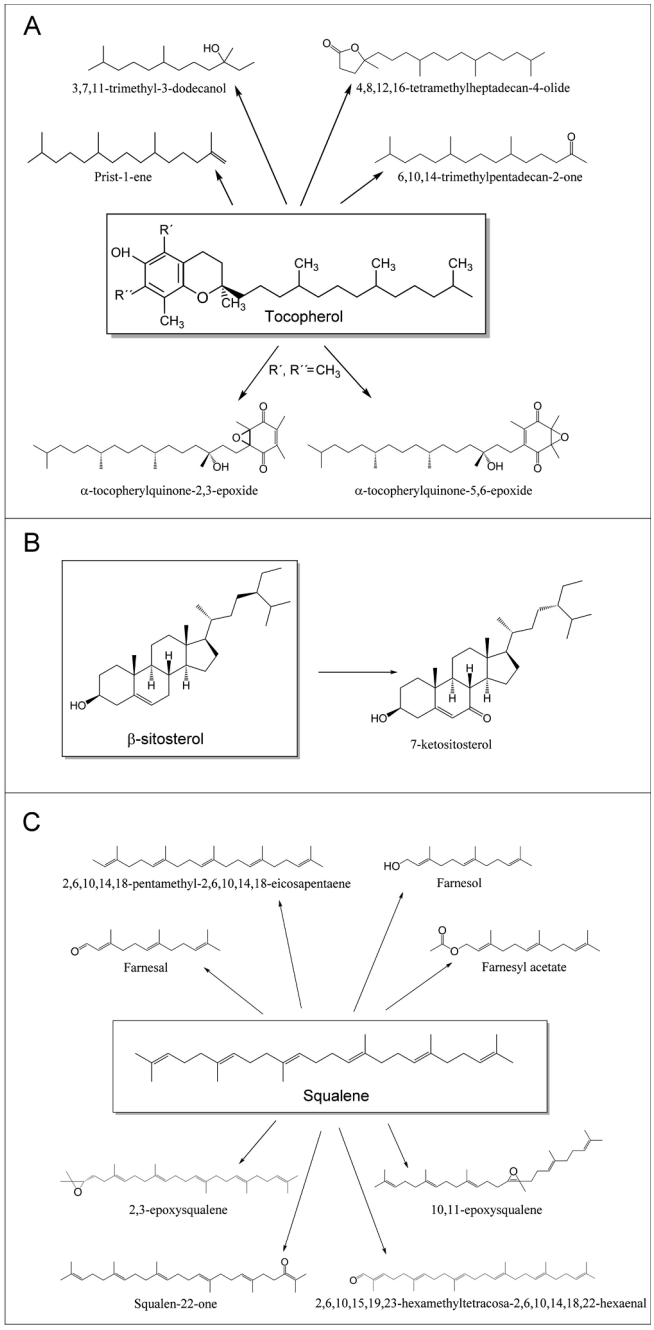

Fig. 2. Detected oxidation compounds derived from: A) tocopherols, B) β-sitosterol and C) squalene.

from day 3 onwards (Table 2 and Fig. S2); the three show increasing abundances until day twelve. Likewise, the abundance of TrMD, which was detected from day 6, shows a growth path to day twelve. The abundance [**DT**] of each one of these four compounds and time **t** under oxidative conditions fit well to equations like [**DT**] = **a** e^{kt}, where **a** is a coefficient specific of each compound, and **k** the formation rate constant. The equations corresponding to each one of the above mentioned tocopherol derivatives, are: [**P**] = 1.0 $e^{0.2t}$ (R = 0.9751, n = 5); [**TrMPD**] = 0.5 $e^{0.3t}$ (R = 0.9740, n = 5); [**TrMD**] = 1.4 $e^{0.3t}$ (R = 0.9808, n = 3); and [**TeMHD**] = 2.7 $e^{0.4t}$ (R = 0.9898, n = 4). From these results it is evident that TeMHD has the highest formation rate among these four tocopherol derivatives.

The presence of these four tocopherol derivatives in the oil indicates that tocopherols have undergone degradation and their presence and abundance are associated with advancing corn oil oxidation. For this reason, they can be considered as corn oil oxidation markers. The detection of P and TrMPD in the original oil in very low abundances corresponds to a basal oxidation level which may occur in many edible oils before submission to oxidative conditions. However, the detection of TeMHD (see Table 2 and Fig. S2) at a very low abundance at day 3 is an indication that the oil has begun its oxidation process. It should be noticed that this lactone has not been detected in unoxidized edible oils such as sunflower oil, virgin and refined soybean oil, linseed oil, and several corn oils (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017; Alberdi-Cedeño, Ibargoitia, & Guillén, 2017). Furthermore, as its abundance increases very quickly throughout the oxidation process, this compound can be considered a good marker of incipient oxidation in edible oils containing tocopherols (see Fig. S2). Finally, the detection of TrMD in the corn oil only occurs after six days under oxidative conditions. For this reason, it can also be considered a corn oil oxidation marker but not from an early stage.

The second of the groups mentioned above includes the compounds coming exclusively from α-T. Two are the members of this group: α-tocopherylquinone-2,3-epoxide (TQ23E) and α-tocopherylquinone-5,6-epoxide (TQ56E) (see Table 2 and Fig. 2A). Their formation from α-T degradation has been described in model systems constituted by α-T, triolein and tripalmitin or by α-T and triolein submitted to thermoxidation or to deodorization simulated conditions respectively (Verleyen, Kamal-Eldin, Dobarganes, Verhé, Dewettinck, & Huyghebaert, 2001; Verhé, Verleyen, Van Hoed, & De Greyt, 2006). The evolution of the abundance of these two compounds follows a different path than to the above mentioned tocopherol derivatives. These are not detected before day 6, reach a maximum on day 9, after which their abundance decreases, probably because these compounds evolve to form others. Their presence in the oil also indicates that an oxidation process has taken place.

In summary, all of these tocopherol derivatives can be considered corn oil oxidation markers and this can be extrapolated to all edible oils containing tocopherols. To the best of our knowledge, this is the first time that these compounds have been proposed as edible oil oxidation markers. It should taken into account that in this same oil the hydroperoxides and their associated conjugated dienic systems (primary oxidation compounds coming from main corn oil components) are not detectable by ^{1}H NMR before day four under the same oxidative conditions (see Fig. S2), and the aldehydes (secondary oxidation compounds coming from main corn oil components) are not detected until day eleven.

3.3.2. Compounds coming from sterols degradation

The detection of sterols derived compounds was much more difficult than that of tocopherol derivatives. Of the various sterol oxidation derivatives described (O'Callaghan, Mc Carthy, & ÓBrien, 2014) only 7-ketositosterol, which is derived from β -sitosterol (the main sterol in this oil), has been detected, but in very small abundance. This compound was detected after 12 days under oxidative conditions, coinciding with an important degree of β-sitosterol degradation (see Table 2 and Fig. 2B and S1B). The formation of 7-ketositosterol in the oxidation of β -sitosterol at different temperatures has been proved previously by several authors (Zhang et al., 2005; Hu et al., 2015).

3.3.3. Compounds coming from squalene degradation

As mentioned before the squalene degradation rate is very slow before day 9. However, as Table 2 shows, some of its derived compounds are already detected on day 9 and others on day 12. The derived compounds found, as Table 2 and Fig. 2C shows, are: four oxygenated derivatives of farnesene; one unsaturated branched hydrocarbon of 25 carbon atoms; and four oxygenated derivatives of squalene, among which there are two epoxides, one ketone and one aldehyde. The formation of these compounds from squalene oxidation has been proved in studies carried out in our laboratory by submitting pure squalene to the same oxidative conditions as here. Of all squalene derivatives, the two epoxy-squalene isomers, squalen-22-one, farnesal and farnesyl acetate were the first formed and the most abundant. Although these compounds were not present initially in this corn oil, some of them have been detected, by using this methodology, in various edible oils not submitted to oxidative conditions (Alberdi-Cedeño, Ibargoitia, Cristillo et al., 2017). Furthermore, 2,3-epoxysqualene has also been identified as an intermediate in the biosynthesis of terpenoids in plants, animals, and fungi (Abe, 2014). For these reasons its presence in edible oils in general could have various origins.

3.3.4. Compounds coming from cyclic dipeptides degradation

None of these compounds could be detected. This could be expected because these antioxidant compounds are in very low abundance in the original oil.

3.4. Evolution with time of compounds coming from corn oil main components oxidation

It is well known that the main corn oil components are triglycerides, having linoleic as main acyl group, followed by oleic, saturated and linolenic acyl groups, this latter in a very small molar percentage, as mentioned in the experimental section. Their oxidation first generates hydroperoxides, also named primary oxidation compounds; these are intermediate compounds that in turn degrade forming different kinds of secondary oxidation compounds. These latter include a great number of small compounds, which are detectable by this technique, among which aldehydes, furan derivatives, alcohols, ketones and lactones are.

3.4.1. Aldehydes

These compounds are the most numerous and abundant secondary oxidation compounds of those detected here. All of them have been previously found in different edible oils submitted to oxidative conditions (Guillén et al., 2005; Guillén & Uriarte, 2012b; Goicoechea & Guillén, 2014). It can be observed in Table S1 that some alkanals, (*E*)-2-alkenals, (*E,E*)-2,4-alkadienals, certain aromatic aldehydes and even some oxygenated *alpha,beta* unsaturated aldehydes are present in this original corn oil, in very low abundance. This indicates again that this oil has a basal oxidation level, which was also evidenced by the presence of two tocopherol derivatives (P and TrMPD), before mentioned.

The abundance of aldehydes increases, as time under oxidative conditions increases. This increase follows a general path of two stages. In the first stage (from day 0 to day 6) the formation rate is much slower than in the second stage (from day 6 onwards) (see Table S1 and Fig. S3). In each one of these stages the abundance fits well with storage time through linear or exponential equations that relate both variables with very high correlation coefficients. It is noteworthy that the point between these two stages occurs after six days under oxidative conditions, that is to say when tocopherols have been degraded to a great extent. Since linoleic is the main acyl group in corn oil, its derived aldehydes are the most abundant. All aldehydes are known oxidation markers and the greater their abundance the greater the oil oxidation

level.

3.4.2. Other secondary oil oxidation compounds

In addition to aldehydes, some furan derivatives, alcohols, ketones, as well as furanones and lactones were also detected in the corn oil after its submission to accelerated storage conditions. All of them are generated in the oxidation of oil main components. They are shown in Table S2, and some of them, such as 2-pentylfuran, 1-octen-3-ol, 3-nonen-2-one, 2,3-octanedione and 5-pentyl-2(5H)-furanone, are well known oil oxidation markers (Guillén et al., 2005; Guillén & Uriarte, 2012b; Goicoechea & Guillén, 2014). The evolution of their abundance, like that of aldehydes, shows two stages of different growth rate. In both stages, abundance fits well with storage time to linear or exponential equations (see Fig. S3). The point between both stages also occurs at day six as in the case of the aldehydes mentioned above.

3.5. Evolution of glycidyl fatty acid esters

As mentioned before, three glycidyl fatty acid esters, GEs, were present in the original oil. These are glycidyl linoleate, glycidyl oleate and glycidyl palmitate. Their abundance seems to increase with time under oxidative conditions until day 9, after which they begin to decline until their total disappearance on day twelve (Table S3 shows this evolution). Their increase could be attributed to heating. It has been described that they are formed from mono- and di-acylglycerides by heating during oil deodorization (Cheng et al., 2016). Their decrease after a certain time under oxidative conditions is in agreement with observations made by Aniołowska and Kita (2015, 2016).

3.6. Comparison of the performance of this methodology in the study of oil oxidation with that of other methodologies that do not require chemical changes in the sample either

In addition to the methodology used here, (DI-SPME-GC/MS), there are others which also provide information on the oxidation status of edible oils, without requiring chemical modification of the sample. One of these involves the study of the oil headspace components, by gas chromatography/mass spectrometry, after their extraction by means of solid phase microextraction (HS-SPME-GC/MS). The other studies the oil sample as a whole by means of ^{1}H NMR spectroscopy.

3.6.1. Performance of HS-SPME-GC/MS

This methodology provides information about the oil oxidation status through the abundance of volatile secondary oxidation compounds. The oxidation markers in this case are the volatile compounds generated in the main oil components oxidation. This has been shown in previous papers (Guillén et al., 2005; Guillén & Uriarte, 2012b; Goicoechea & Guillén, 2014). Nevertheless, all these compounds are also detected by DI-SPME-GC/MS as has been described in point 3.4. Furthermore, it should be noticed that extraction by SPME of a compound from a headspace (HS) requires greater abundance of the compound in the oil than does its extraction from the oil matrix directly by means direct immersion (DI). For this reason, volatile compounds in very small abundance in the oil can be detected by using direct immersion-solid phase microextraction (DI-SPME) but not by headspace solid phase microextraction (HS-SPME). Likewise, in the study of an oil oxidation process the minor volatile components newly formed are detected slightly earlier by direct immersion than through the study of the oil headspace. In addition it should be remembered that DI-SPME-GC/MS, in addition to the above mentioned secondary oxidation compounds, also provides information about the oil antioxidant components and of some of their derived compounds, as shown in points 3.2 and 3.3.

3.6.2. Performance of ^{1}H NMR spectroscopy

This technique provides qualitative and quantitative information not only of the oil main components (molar percentage of the several kinds of acyl groups), but also of their primary and secondary oxidation derivatives. The information of primary oxidation compounds refers to total hydroperoxides and to their *Z,E*- and *E,E*-conjugated dienic systems. The information of secondary oxidation compounds refers mainly to keto- and hydroxy-dienes as well as to epoxy derivatives and to several kinds of aldehydes (Martin-Rubio, Sopelana, Ibargoitia, & Guillén, 2018).

Of all those compounds which may be studied by ^{1}H NMR spectroscopy, DI-SPME-GC/MS is only able to provide information of some secondary oxidation compounds as indicated in point 3.4. However, as mentioned above, this latter technique provides information of the oil antioxidant components and of some of their derived compounds (points 3.2 and 3.3), which cannot be obtained directly from ^{1}H NMR. Furthermore, the information provided by this latter technique in most cases does not refer to individual compounds but to groups of compounds having the same functional group and protons with similar electronic environment (Guillén & Ruiz, 2004; Guillén & Ruiz, 2005; Martin-Rubio et al., 2018). In addition, its sensitivity is low, for which reason the compounds are only detected from a certain abundance level onwards.

In summary, HS-SPME-GC/MS only provides information about volatile secondary oxidation compounds derived from oil main components. ^{1}H NMR provides information concerning main oil components, and their primary and secondary oxidation compounds. DI-SPME-GC/MS provides information about oil antioxidant components, some compounds coming from antioxidant components oxidation, and some secondary oxidation compounds coming from main oil component oxidation.

4. Conclusions

Study by direct immersion-solid phase microextraction followed by gas chromatography/mass spectrometry of corn oil and of this oil submitted to storage accelerated conditions provides a great deal of information concerning its volatile and less volatile minor components. Among these latter there are very important antioxidant corn oil components, such as tocols, sterols, squalene and cyclic dipeptides that play an essential role in the oil oxidation process. This methodology provides information about the degradation rate of these compounds and also about the identity and formation rate of compounds coming from their degradation during storage. The compounds formed in the degradation of tocopherols are proposed here, for the first time, as new oxidation markers of corn oil and of any edible oils containing tocopherols. Among these are 3,7,11-trimethyl-3-dodecanol and 4,8,12,16-tetramethylheptadecan-4-olide, whose formation in edible oils from tocopherols, is described for the first time in this paper. The latter can be considered a very good corn oil oxidation marker because it is formed very early after oil subjection to oxidative conditions and because it has a high formation rate. The formation and evolution of secondary oxidation volatile compounds derived from main corn oil components, all them well known oxidation markers, can also be analyzed by this methodology. The abundance of these becomes noticeable coinciding with the effective decline of those compounds having antioxidant ability. Likewise, this methodology enables the detection and study of the evolution, under these storage conditions, of contaminants present in the original oil such as various glycidyl esters. The usefulness of this technique for the study of edible oil oxidation processes is shown for the first time, providing new information which would be difficult, if not impossible, to obtain by other methods.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.04.001.

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