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Effect of garlic essential oil on sunflower oil oxidative stability during accelerated storage studied by FTIR spectroscopy

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ABSTRACT

Garlic essential oil (GEO) was added to sunflower oil at different concentrations (0.005%, 0.5%, 10%) and enriched and non-enriched oils were subjected to accelerated storage (70 °C). Oil samples were studied daily by Fourier Transform Mid-Infrared (FTIR) spectroscopy. The changes in the frequency values and absorbance of specific bands of the spectra allowed to shed light on the effect of GEO on oil oxidative stability, in a very simple, fast and accurate way, providing information on the degradation of *cis*-double bonds of unsaturated acyl groups (band near 3008 cm^{-1}), as well as on the generation of oxidation compounds like hydroperoxides (band near 3469 cm^{-1}) and aldehydes (band near 1746 cm^{-1}). Moreover, GEO composition was studied by Proton Nuclear Magnetic Resonance spectroscopy (^1H NMR) and by Solid-Phase Microextraction coupled with Gas Chromatography-Mass Spectrometry (SPME-GC/MS). The main GEO components identified were sulphur-containing compounds (diallyl-disulfide, diallyl-trisulfide, methyl-allyl-disulfide …). The addition of GEO at 0.005% did not affect oil oxidative stability; at 0.5%, it provoked a decrease in oil oxidative stability; and the addition of 10% GEO increased it, causing remarkable differences in the oxidation process compared to the other oils under the same storage conditions. The present study evidences that under accelerated storage conditions the antioxidant efficacy of GEO depends on the concentration added to sunflower oil. This study also underscores the need for careful evaluation of the effect of potential antioxidants, emphasizing the usefulness of FTIR to follow the changes occurring in edible oils during oxidation under accelerated storage conditions.

1. Introduction

In recent decades, lipid oxidation reactions that can occur during food processing and storage have received much attention due to their economic and health implications. It is well known that lipid oxidation involves very complex reactions due to the incorporation of oxygen into the unsaturated acyl chains of triglycerides (Frankel, 2005). In brief, according to the generally accepted scheme, firstly hydroperoxides supported in chains having also *cis,trans*- or *trans,trans*-conjugated dienes are formed, also known as primary oxidation compounds. These are very unstable and easily decompose into secondary oxidation compounds of very different nature, volatile or not, including aldehydes, ketones, alcohols, acids and furans. Some of these oxidation compounds could impair not only the nutritional and sensory quality of foods, but also their safety. It should be noted that it is well known that the nature and concentration of lipid oxidation compounds formed depend on several factors, such as food lipid nature, the degradative conditions and the presence of compounds that can show antioxidant or prooxidant activity (Frankel, 2005; Martínez-Yusta et al., 2014).

Thus, with the growing global demand for healthier foods, it is considered of great interest to find ways to reduce lipid oxidation and the formation of potentially harmful oxidation compounds. It must be noted that the latter can be absorbed through the diet and have been linked to the development of inflammatory diseases, as well as cancer, atherosclerosis, and ageing, among others (Vieira et al., 2017). In order to reduce lipid oxidation in foods, in the case of edible oils aimed to be consumed directly or taking part of a food product as an ingredient, one of the main studied strategies is the addition of antioxidants. Furthermore, the growing consumer concern about the possible toxicity of synthetic antioxidants (Amorati et al., 2013; Brewer, 2011), has led the food industry and researchers to focus on those of natural origin. Most of them are obtained from vegetable sources and could be added to food either as pure compounds, or present in mixtures like plant extracts or essential oils. The latter are liquid mixtures of volatile compounds

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obtained from plants. In recent years the use of essential oils is being considered as an interesting possible option to be added directly to edible oils or fats, to foods containing them as ingredients, or in active packaging and edible coatings, in order to delay lipid oxidation and extend food shelf life (Amorati et al., 2013; Tsitlakidou et al., 2023).

Garlic (*Allium sativum*) is a plant native to central Asia, whose bulbs contain 1%–2% of essential oil on a dry basis. It has been consumed worldwide since ancient times, not only as a culinary ingredient, but also in medicine for the prevention and treatment of several diseases (Rivlin, 2001). Garlic essential oil (GEO) contains a variety of organosulfur compounds, being the most abundant diallyl-disulfide, to which several potential biological activities have been attributed, such as antioxidant one due to their ability to reduce hydroperoxides (Amorati et al., 2013; Banerjee et al., 2003; Ezeorba et al., 2022; Huang et al., 2023; Verma et al., 2023). Moreover, it is remarkable that several recent reviews have focused on the potential health and therapeutic benefits of GEO, including antimicrobial, cardioprotective, anti-inflamatory, anti-cancer, anti-diabetic and anti-hyperlipidemic activities, among others (Ezeorba et al., 2022; Huang et al., 2023; Verma et al., 2023).

It must be noted that GEO volatile constituents are not originally present in the plant. They are generated during chopping or crushing of garlic cloves by the action of the enzyme allinase, which transforms the alliin (sulfoxide derivative of the amino acid cysteine) into allicin, which is unstable and quickly gives rise to the above-mentioned sulphur-containing compounds, among others (Amorati et al., 2013). According to Olivas-Méndez et al. (2022) the addition of 1% GEO to beef hamburgers stored at 4 °C not only delayed lipid oxidation, determined by the quantification of thiobarbituric acid reactive substances (TBARS), but also reduced the growth of aerobic bacteria, mold and yeast, improving microbiological quality. Furthermore, hamburgers enriched with 1% GEO showed a better sensory acceptance by consumers, than control hamburgers. On the contrary, Sun et al. (2000) claimed that the addition of 0.006% garlic oil to Chinese-style heat-dried sausages did not afford any significant antioxidant effect, studied using the same methodology (TBARS). It must be noted that the limitations of this classical methodology are well known, as it does not provide specific information about the nature of the oxidation compounds generated (Barriuso et al., 2013; Frankel, 2005). Thus, several authors have recommended to carry out the evaluation of the activity of potential antioxidants under a holistic perspective, this is, under different conditions of oxidation and using specific methodologies able to define what products are formed and inhibited by the presence of antioxidants in each case (Amorati et al., 2013; Frankel, 1993; Frankel & Meyer, 2000).

Fourier Transform Mid-Infrared (FTIR) spectroscopy is a non-destructive, clean, simple and fast technique (1–2 min), that is nowadays considered a green analytical technique. FTIR has been commonly used for structurally elucidating organic molecules on the basis of characteristic fundamental vibrations of some functional groups, and its usefulness for the characterization of food lipids and the study of their oxidative stability is well known (Guillén & Cabo, 1997, 1999, 2004; Li et al., 2019; Navarra et al., 2011; Van de Voort et al., 1994). FTIR delivers sound results with minimal sample preparation, and, as the sample is studied directly without any chemical modification, the risk of artifact formation is minimized. Its main limitation is its inability to provide full structural information on lipid components in the way that techniques like Nuclear Magnetic Resonance (NMR) spectroscopy and Mass Spectrometry (MS) can. Nevertheless, it must be noted that FTIR provides several advantages in comparison with other traditional methods of evaluating lipid oxidation, such as the above-mentioned TBARS test. This latter test provides only partial information on the oxidative process, as the compound measured (malondialdehyde) is mainly derived from linolenic chains (C18:3ω3) and is only one of the many possible secondary oxidation products formed; in addition, other compounds not resulting from the oxidation process can also contribute to TBARS values (Barriuso et al., 2013; Frankel, 2005).

In this context, this study first characterized the composition of GEO. For this purpose, its volatile components were identified by Solid-Phase Microextraction followed by Gas Chromatography-Mass Spectrometry (SPME-GC/MS), and its liquid phase was also studied by Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR). The aim of this study was to evaluate the effect of the addition of GEO at 0.005%, 0.5% and 10% on sunflower oil oxidative stability subjected to accelerated storage conditions by means of FTIR spectroscopy. To obtain further complementary information on the oxidation compounds generated during storage, some enriched and non-enriched oil samples were also studied by ^{1}H NMR.

2. Materials and methods

2.1. Samples and oxidative conditions

Steam-distilled Garlic Essential Oil (GEO) was acquired from a local company supplying ingredients for the food industry, and refined sunflower oil was acquired from a local supermarket. GEO was added to sunflower oil at 0.005%, 0.5% and 10%. Enriched and non-enriched sunflower oils were submitted to accelerated storage conditions. For this purpose, several 10 g samples of sunflower oil held in crystal Petri-dishes of 80 mm diameter and 15 mm high, were placed in a convection oven (Memmert GmbH + Co. KG, Schwabach, Germany), with circulating air and without being stirred. Oven temperature was maintained at 70 °C with a stability of ±0.5%. The Petri-dishes were introduced into the oven without their lids to facilitate exposure to the circulating air. The experiments were carried out in duplicate and samples were studied daily by FTIR, until polymerization of the samples.

Some preliminary studies were conducted to determine the concentration levels to be applied. Sunflower oil was mixed with 0.001%, 0.005%, 0.05%, 0.1%, 0.5%, 5% and 10% of GEO, and subjected to accelerated storage conditions for 7 days. This period was selected because it is when the highest concentration of hydroperoxides is detected in non-enriched sunflower oil (Goicoechea & Guillen, 2010; Guillén & Cabo, 1999). Additionally, 16 panelists evaluated the acceptability of odour and taste of these enriched oils at day 0 and oil samples containing up to a 0.05% enrichment level were considered acceptable. Taking into account the information provided by FTIR and sensory analysis results, the concentrations 0.005%, 0.5% and 10% were selected for further study. Although sunflower oil enriched with 0.5% and 10% GEO would not be acceptable to be consumed directly, they could be considered of interest as minor ingredients in the formulation of food products.

2.2. Infrared Spectra Acquisition

The infrared spectra were recorded on a FTIR Bruker Vector 33 (Bruker Optics GmbH & Co. KG, Ettlingen, Germany) interfaced to a personal computer operating under Opus NT software (version 2.0). A film of a small amount of sample (approximately 2 μL) was placed between two disks of KBr (32 × 3 mm), avoiding the presence of air, and screwing the screws of the sample holder as far as possible so that the path length was constant for all of the samples, as in previous studies (Guillén & Cabo, 1999). Duplicate spectra were collected daily until the sample became so viscous that it was impossible to put a film of the sample between the KBr disks. All spectra were recorded from 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}. For each spectrum 32 interferograms were co-added before Fourier transformation and zero-filled to give a data point spacing of 1.9 cm^{-1}. The measurement accuracy in the frequency data was better than 0.01 cm^{-1} because of the laser He–Ne internal reference of the instrument. The frequency value for each band was obtained automatically by the equipment software. The assignment of the bands to the specific functional group vibration mode was made by a comparison to previous studies of edible fats and oils (Guillén & Cabo, 1999; Van de Voort et al., 1994). It should be noted that the registration of the spectra does not require any modification or

manipulation of the samples, and that the spectra and the derived data are very similar in the duplicated experiments, the standard deviations being very small. Fig. 2 of oil FTIR spectra was plotted at a fixed value of absolute intensity to be valid for comparative purposes.

2.3. Study of the headspace composition of GEO

2.3.1. Extraction of volatile components by solid phase microextraction (SPME)

Vials containing 0.03 mg of GEO were introduced into a water bath maintained at 50 °C. After a period of sample equilibration (15 min), a fibre coated with DVB/CAR/PDMS (Divinylbenzene/Carboxen/Polydimethylsiloxane, 1 cm long, 50/30 μm film thickness), acquired from Supelco (Merck, Darmstadt, Germany), was inserted into the headspace of the sample and was maintained for 60 min. The selection of the fibre type and of the extraction operating conditions was previously studied in our laboratory. The usefulness of DVB/CAR/PDMS fiber for the study of garlic flavor components was also evidenced by Lee et al. (2003).

2.3.2. Gas Chromatography/Mass Spectrometry (GC-MS) study

The fibre was desorbed for 10 min in the injection port of a Hewlett-Packard (HP) 6890 Series II Gas Chromatograph equipped with an HP 5973 mass-selective detector and a computer operating with the ChemStation program (Hewlett-Packard, Palo Alto, CA, USA). The column used was a fused silica capillary column (60 m length × 0.25 mm inner diameter × 0.25 μm film thickness; HP) coated with a nonpolar stationary phase (HP-5MS, 5% phenylmethylsiloxane). The operating conditions were as follows: the oven temperature was set initially at 50 °C (5 min hold), then increased at 4 °C/min to 280 °C (2 min hold); the temperatures of the ion source and the quadrupole mass analyser were kept at 230 and 150 °C respectively; helium was used as carrier gas at a pressure of 16.5 psi; the injector and detector temperatures were held at 220 and 280 °C respectively; splitless mode was used for injection, with a purge time of 1.5 min. Mass spectra were recorded at an ionisation energy of 70 eV, with data acquisition in Scan mode. After the first desorption, the fiber was routinely desorbed for a second time in order to determine if the first process was complete.

Many components were identified using standards acquired commercially (Sigma-Aldrich, Burlington, MA, USA), which are asterisked compounds shown in Table 1. Many other components were tentatively identified, taking into account retention times and more than 85% agreement with mass spectra of a commercial library (Wiley W9N08.L, Mass Spectral Database), as in previous studies (Guillen & Goicoechea, 2008). Semi-quantification of the components was based on the area count of the base peak of the mass spectrum of each compound divided by 10^6. The base peak is the most intense peak (intensity 100%) in the mass spectrum of a compound. The results were obtained as average values of two determinations.

2.4. Study by Proton Nuclear Magnetic Resonance spectroscopy (^1H NMR)

GEO and some enriched and non-enriched oils were analyzed by ^1H NMR spectroscopy using a Bruker Avance 400 spectrometer operating at 400 MHz, as in previous studies (Goicoechea & Guillen, 2010; Martínez-Yusta et al., 2014). To prepare the samples, 175 μL of each oil were mixed with 425 μL of deuterated chloroform ($CDCl_3$) in a 5 mm diameter ^1H NMR tube. The $CDCl_3$ contained a small amount (0.2%) of non-deuterated chloroform and 0.03 % of tetramethylsilane, used as internal reference (Eurisotop, Paris, France). Each sample was analyzed in duplicate. The relaxation delay and acquisition time were set to allow complete relaxation of the proton nuclei. This ensured that signal areas were directly proportional to the number of protons generating them. The spectra shown in Fig. 4 were plotted at a fixed value of absolute intensity to be valid for comparative purposes using MNova program (Mestrelab Research, Santiago de Compostela, Spain).

Table 1
Volatile compounds detected in the headspace of garlic essential oil (GEO), together with their mean abundances, expressed as area counts of their mass spectra base peak (Bp) divided by 10^6.

Compound name (Molecular weight)	Bp	Abundance
Aliphatic sulphur derivatives		
Metanethiol (48)	47	1.89 ± 0.12
2-Propene-1-thiol (allyl-thiol) (74)	74	73.94 ± 3.66
Methyl-allyl-sulfide (88)	88	130.71 ± 8.12
Dimethyl-disulfide (88)	94	78.31 ± 4.81
Allyl-isopropyl-sulfide (116)	74	0.45 ± 0.05
Diallyl-sulfide (114)*	73	238.93 ± 11.18
Methyl-propyl-disulfide (122)	80	0.56 ± 0.04
Methyl-allyl-disulfide (120)*	120	505.75 ± 28.21
Dimethyl trisulfide (126)	126	74.70 ± 4.12
Allyl-propyl-disulfide (148)	148	31.74 ± 2.56
Diallyl-disulfide (146)*	41	1009.94 ± 42.16
Methyl-allyl-trisulfide (152)	87	232.70 ± 9.23
Dimethyl-tetrasulfide (158)	158	3.96 ± 0.16
Diallyl-trisulfide (178)*	113	549.44 ± 19.23
Methyl-allyl-tetrasulfide (184)	120	140.82 ± 7.65
Diallyl-tetrasulfide (210)	146	166.90 ± 8.36
Cyclic sulphur derivatives		
1,2- Dithiacyclopentane (1,2-dithiolane) (106)	106	30.47 ± 2.74
Tetrahydro-2,5-dimethylthiophene (116)	101	0.73 ± 0.04
Trithioformaldehyde (1,3,5-Trithiane) (138)	138	185.11 ± 9.22
3-Vinyl-1,2-dithiocyclohex-4-ene (144)	144	100.36 ± 4.61
3-Vinyl-1,2-dithiocyclohex-5-ene (144)	72	130.40 ± 6.13
2-(2-Thio-4-pentenyl)-1-thio-cyclohex-5-ene (186)	145	11.85 ± 0.13
3-(2,3,4-Trithio-6-heptenyl)-1-thio-cyclohex-5-ene (250)	145	43.62 ± 3.41
Hydrocarbons		
1-Propene (42)*	41	119.06 ± 6.24
1,5-Hexadiene (82)*	67	15.82 ± 0.90
2-Methyl-1,3-pentadiene (84) (or isomer)	67	2.01 ± 0.07
3-Methyl-1,3-pentadiene (84) (or isomer)	67	1.30 ± 0.03
4-Methyl-cyclopentene (82)	67	2.20 ± 0.10
Cyclohexane (84)*	56	0.28 ± 0.01
Toluene (methylbenzene) (92)*	91	0.45 ± 0.02
Aldehydes		
2-Propenal (56)*	56	5.03 ± 0.23
5-Hydroxy-methyl-furfural (126)*	97	0.53 ± 0.04
Alcohols		
2-Propen-1-ol (58)*	57	13.43 ± 0.42

2.5. Statistical analysis

All samples were prepared and analyzed twice by FTIR in individual experiments, which were carried out in duplicate ($n = 4$). The statistical significance of the differences between the frequency values of some FTIR bands of the spectra of enriched and non-enriched oils submitted to accelerated storage were studied using a one-way analysis of variance with a statistical acceptance level of $P < 0.05$ (SPSS v.28, IBM Corporation, NY, USA).

3. Results and discussion

Firstly, the composition of GEO was studied by SPME-GC/MS and ^1H NMR and secondly, the effect of its addition at 0.005%, 0.5% and 10% on sunflower oil oxidative stability submitted to accelerated storage conditions was evaluated by FTIR.

3.1. Characterization of garlic essential oil composition

Table 1 shows the volatile compounds identified in the headspace of GEO by SPME-GC/MS, together with their mean abundances. Most of them were sulphur-containing derivatives, either aliphatic or cyclic, in agreement with other studies (Banerjee et al., 2003; El-Sayed et al.,

2017; Santhosha et al., 2013). The most abundant volatile components present in GEO were diallyl-disulfide, diallyl-trisulfide and methyl-allyl-disulfide, followed by diallyl-sulfide and methyl-allyl-trisulfide. Among aliphatic sulphur-containing derivatives, the following were identified: three monosulfides, five disulfides, three trisulfides and three tetrasulfides, being disulfides the most abundant ones. Many of these sulphur derivatives, especially sulfides having an allyl group, are responsible for the characteristic smell and taste of garlic. It must noted that several health protective effects have been attributed to some of them (Santhosha et al., 2013). Furthermore, as can be observed in Table 1, in the headspace of GEO four aliphatic hydrocarbons, two cyclic hydrocarbons, one aromatic hydrocarbon, two aldehydes and one alcohol were also identified. Most of them were detected in very low abundances, except for 1-propene.

As essential oils are liquid mixtures of volatile compounds (Amorati et al., 2013), for confirmatory purposes it was considered of interest to study also the liquid phase of GEO by ^{1}H NMR. Thus, Fig. 1 shows the ^{1}H NMR spectrum of GEO, and the assignment of the corresponding proton signals is provided in Table 2 (Hile et al., 2004; Sayer et al., 2023). The main proton signals were related to the above-described sulphur derivatives, in agreement with the results obtained by SPME-GC/MS. These main signals were located in two regions of the spectrum: from 3.1 to 3.7 ppm, where doublets due to thioallylic protons CH_2=CH-C**H**$_2$-S- were observed; and from 5 to 6 ppm, where multiplets due to olefinic protons C**H**$_2$=C**H**- appeared. These main proton signals were related to the presence in GEO of compounds having "allyl-sulfide" structures, such as diallyl-disulfide, methyl-allyl-disulfide, diallyl-trisulfide, diallyl-sulfide and methyl-allyl-trisulfide, among others. In addition, in the region from 2.0 to 2.7 ppm some singlets were also detected, due to the thiomethylic protons C**H**$_3$-S-, that can be supported on compounds like methyl-allyl-disulfide or dimethyl-disulfide, among others. These results obtained by ^{1}H NMR on the composition of GEO are in agreement with those provided on other GEO from different origins, using the same technique (Hile et al., 2004; Sayer et al., 2023).

3.2. FTIR study on the effect of the addition of GEO on sunflower oil oxidative stability during accelerated storage

It is well known that FTIR spectroscopy is a very useful tool for the study not only of the composition of edible oils and fats, but also of the changes occurring during their oxidation under accelerated storage conditions. It has been shown that the evolution of the mid-infrared spectra of oils throughout the oxidation process at these temperatures follows a general pattern, and that the differences between different samples are due to the speed or magnitude of the changes produced (Guillén & Cabo, 1997, 1999, 2004; Navarra et al., 2011; Van de Voort et al., 1994). In this context, to shed light on the effect of the addition of GEO on sunflower oil oxidative stability, special attention was paid to the changes occurring in the frequency values and absorbance of three bands of the spectra, which are known to provide information on the generation of oxidation compounds like hydroperoxides (band near 3469 cm^{-1}) and aldehydes (band near 1746 cm^{-1}), as well as on the degradation of *cis*-double bonds of unsaturated acyl groups (band near 3008 cm^{-1}). Fig. 2 shows the FTIR spectra of non-enriched sunflower oil and of sunflower oil enriched at 0.005%, 0.5% and 10% of GEO, after being submitted to accelerated storage at 70 °C for several days. The evolution during storage of the frequency values of the above-mentioned three FTIR bands in non-enriched and enriched oils is shown in Fig. 3.

3.2.1. FTIR band near 3469 cm^{-1}

3.2.1.1. Changes in the spectra of non-enriched sunflower oil during storage. As can be observed in the spectra of non-enriched oil at day 0 (see Fig. 2a), in the region between 3600 and 3100 cm^{-1} there was a small narrow band near 3469 cm^{-1}, which is related to the overtone of the glyceride ester carbonyl absorption (Guillén & Cabo, 1997, 1999; Van de Voort et al., 1994). Under these oxidative conditions, from day 5 onwards this band widened, became more intense and its frequency value started to decrease, reaching the lowest value at day 7 (3429.20 cm^{-1}, see Fig. 3a). This change in the intensity and frequency value of this band was due to its overlapping with new absorptions near 3445 cm^{-1} caused by hydroperoxides generated in the oxidation process, in agreement whit other authors (Van de Voort et al., 1994). These well-known primary oxidation compounds are derived from the oxidation of unsaturated linoleic acyl groups (C18:2ω6) under mild oxidative conditions (Frankel, 2005). The period that elapses from the beginning of the oxidation process until the frequency of this band began to decrease, or in other words, in which the frequency remained close to 3469 cm^{-1} although some broadening was observed, is called the First

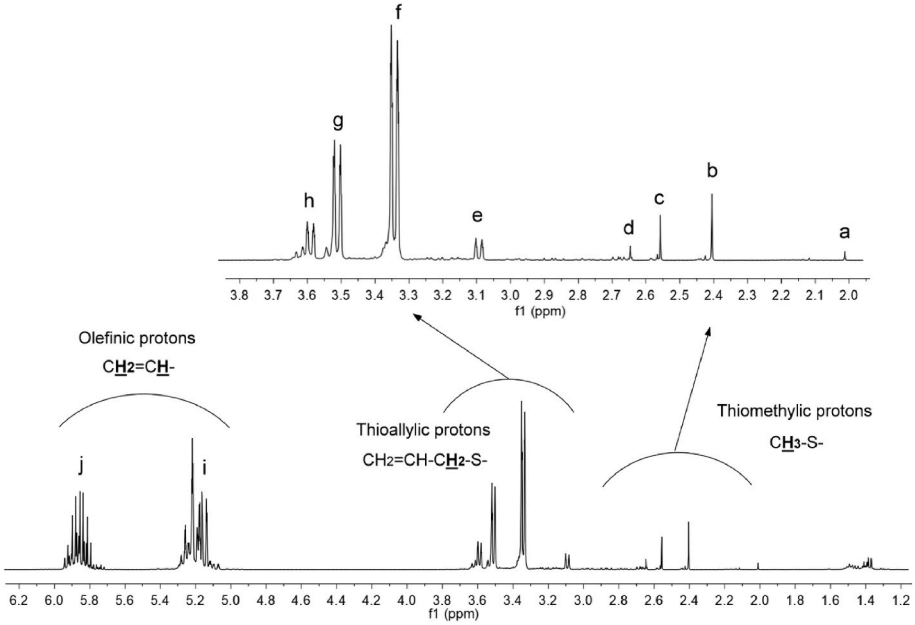

Fig. 1. ^{1}H NMR spectrum of garlic essential oil (GEO). Signal letters agree with those in Table 2.

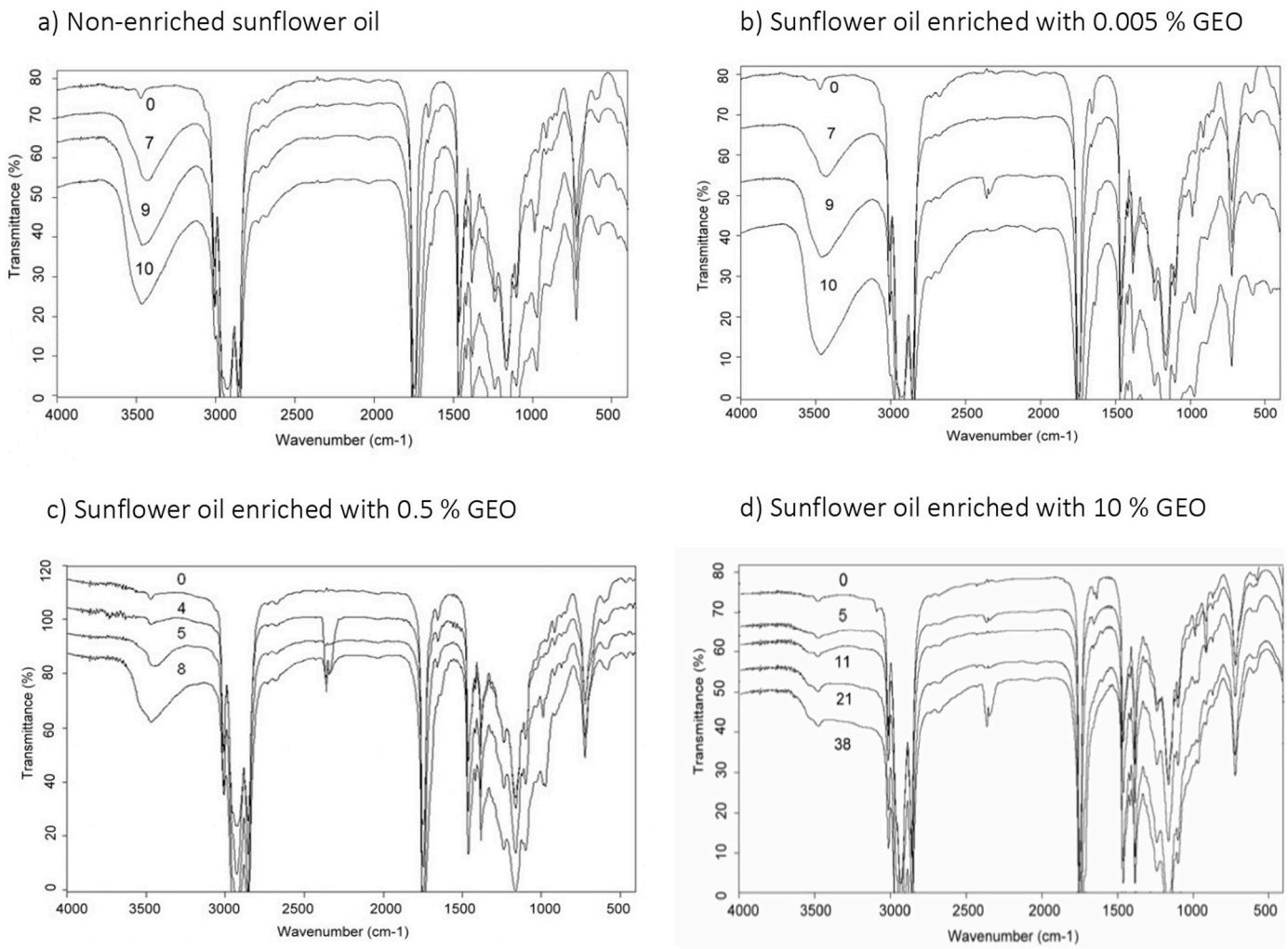

Fig. 2. Fourier transform infrared spectra of **a)** non-enriched sunflower oil and sunflower oil enriched with GEO at **b)** 0.005 %, **c)** 0.5 % and **d)** 10 % of GEO submitted to accelerated storage.

Period (FP, days 0–5 in non-enriched oil) (Guillén & Cabo, 1999).

In non-enriched sunflower oil the frequency of this band remained around 3429 cm^{-1} as long as there was a certain concentration of hydroperoxides in the sample. From day 9 onwards, these were transformed into secondary oxidation compounds and the frequency of this band increased and returned to the initial values (see Fig. 3a). Also contributing to this shift was the appearance and increase in intensity of a shoulder near 3530 cm^{-1}, related to the formation of alcohols among the secondary oxidation compounds (this shoulder was incipient at day 7) (Van de Voort et al., 1994). This period in which hydroperoxides were present in appreciable proportions, this is from day 6–8, is called the Second Period (SP). It must be noted that before the end of the FP a band appeared near 987.50 cm^{-1}, which remained until the end of the SP (see day 7 spectrum in Fig. 2a). This band, which is associated with the bond vibrations of the CH *cis,trans*- and/or *trans,trans*-conjugated dienes of hydroperoxides, reached the maximum frequency value on day 7 at 988.20 cm^{-1}(Van de Voort et al., 1994). This fact evidenced that the generated hydroperoxide groups were related to conjugated double bonds, in agreement with previous studies on sunflower oil submitted to the same oxidative conditions carried out by FTIR (Guillén & Cabo, 1999) and also by ^{1}H NMR (Goicoechea & Guillen, 2010). The Third Period (TP) describes the time interval between the return of the frequency of this band near 3469 cm^{-1} towards the original values and the total polymerization of the non-enriched sunflower oil, this is days 9–10 (see Fig. 3a). It must be noted that although the frequency value changed, this band remained broadened.

Therefore, the duration of the First Period could be considered as a measure of the oxidative stability of the oil: the shorter the FP, the lower the oxidative stability of the oil and the faster the formation of hydroperoxides. In the case of this non-enriched sunflower oil, the FP duration would be 5 days. Previous FTIR studies have shown differences on the oxidative stability of edible oils of different composition, as the duration of these FP, SP and TP intervals, depended not only on the proportions of saturated, mono- and polyunsaturated acyl groups, but also on the presence or absence of minor compounds showing antioxidant activity (Guillén & Cabo, 1999, 2004).

3.2.1.2. Changes in the spectra of sunflower oils enriched with GEO during storage. Regarding the changes occurring in this band near 3469 cm^{-1} during the storage of sunflower oil **enriched with 0.005% GEO**, as can be observed in Fig. 2b, they were very similar to those described above for non-enriched oil. The intensity and frequency value of this band remained unaltered until day 5. However, from day 5 onwards, this band broadened, became more intense, and its frequency value started to decrease due to the formation of hydroperoxides. As can be observed in Fig. 3a, this band reached a minimum frequency value of 3429.75 cm^{-1} at day 7, similarly to that observed in the spectrum of non-enriched oil at the same day of storage. It must be noted that at the end of the FP the above-mentioned band near 987.5 cm^{-1} also appeared, which is associated with the bond vibrations of the *cis,trans*- and *trans,trans*-

Table 2
Chemical shift assignments and multiplicities of the ^{1}H NMR signals in $CDCl_3$ of the main protons of sulfides present in GEO (Hile et al., 2004; Sayer et al., 2023). The signal letters agree with those given in Fig. 1.

Signal	Chemical shift (ppm)	Multiplicity	Functional group	
			Type of protons	Compound
Thiomethylic protons				
a	2.04	s	C**H**$_3$-S-	Methyl-allyl-sulfide
b	2.42	s	C**H**$_3$-S-	Methyl-allyl-disulfide
	2.44	s	C**H**$_3$-S-	Dimethyl-disulfide
c	2.55	s	C**H**$_3$-S-	Methyl-allyl-trisulfide
	2.56	s	C**H**$_3$-S-	Dimethyl-trisulfide
d	2.65	s	C**H**$_3$-S-	Methyl-allyl-tetrasulfide
	2.67	s	C**H**$_3$-S-	Dimethyl-tetrasulfide
Thioallylic protons				
e	3.10	d	CH_2=CH-C**H**$_2$-S-	Diallyl-sulfide
f	3.36	d	CH_2=CH-C**H**$_2$-S-	Diallyl-disulfide
g	3.52	d	CH_2=CH-C**H**$_2$-S-	Diallyl-trisulfide
h	3.60	d	CH_2=CH-C**H**$_2$-S-	Diallyl-tetrasulfide
Olefinic protons				
i	5.03–5.30	m	C**H**$_2$=CH-	All allylic sulfides
j	5.68–5.95	m	CH_2=C**H**-	“

Abbreviations: s, singlet; d, doublet; m, multiplet.

conjugated olefinic groups CH of hydroperoxides (see day 7 spectrum in Fig. 2b). As these primary oxidation compounds degraded into secondary ones, the frequency of this band returned to original values at 3466.95 cm^{-1}, although it remained broadened. Thus, the duration of the oxidation process periods was the same as in the non-enriched oil: FP days 0–5, SP days 6–8 and TP days 9–10. These results suggest that the addition of 0.005% GEO to sunflower oil did not affect its oxidative stability during accelerated storage.

As for the storage of sunflower oil **enriched with 0.5% GEO**, some differences can be observed in comparison with previous samples. The first one is that this oil polymerized earlier, at day 8 instead of day 10. As it can be observed in the spectra of Fig. 2c, the band near 3469 cm^{-1}, associated with the overtone of the carbonyl group absorption of triglyceride esters, widened and became more intense from day 4 onwards, this is, one day earlier than the non-enriched oil and the oil enriched with 0.005% GEO. It is remarkable that during the storage of this oil enriched with 0.5% GEO the intensity of this band was always lower than that of the non-enriched oil and the oil enriched with 0.005% GEO (compare Fig. 2c with Fig. 2a and b). As can be observed in Fig. 3a, at day 4 its frequency started to decrease due to the formation of hydroperoxides, reaching a minimum of 3449.43 cm^{-1}. It must be noted that this value was higher than the minimum value near 3429 cm^{-1} reached in the above-described non-enriched oil and oil enriched with 0.005% GEO at day 7. These results regarding the frequency value and intensity of this band evidence that during the storage of sunflower oil enriched with 0.5% GEO lower amounts of hydroperoxides were formed, in comparison with non-enriched oil and the oil enriched with 0.005% GEO.

As these primary oxidation compounds degraded into secondary ones, at day 7 the frequency of this band returned to original values at 3469.60 cm^{-1}, although it remained broadened. Thus, the duration of the above-described periods in this oxidation process would be somewhat different from that of the non-enriched oil and the oil enriched with 0.005% GEO: FP days 0–4, SP days 5–6 and TP days 7–8. As mentioned earlier, the duration of the FP could be considered a measure of the oxidative stability of the oil, as a shorter FP indicates lower oxidative stability and faster hydroperoxide formation. Therefore, the results on the evolution of this band near 3469 cm^{-1} during storage suggest that the addition of 0.5% GEO to sunflower oil provoked a decrease in the oxidative stability of the oil during accelerated storage conditions. It must be noted that during the storage of this oil enriched with 0.5% GEO at the end of the FP the above-mentioned band near 987.5 cm^{-1} also appeared, which is associated with the bond vibrations of the *cis,trans*- and *trans,trans*-conjugated olefinic groups CH of hydroperoxides (see day 5 spectrum in Fig. 2c).

Fig. 2d shows some of the FTIR spectra obtained during the accelerated storage of sunflower oil samples **enriched with 10% GEO**. Unlike previous studies on non-enriched and 0.005% and 0.5% GEO enriched oils, in which the oxidation process concluded within 8–10 days due to oil polymerization, in the presence of 10% GEO samples were studied until day 21 of storage. Afterwards they became too viscous and they were totally polymerized by day 39. Thus, the presence of this high concentration of GEO induced substantial changes in the mechanism of the oxidation process occurring under accelerated storage conditions and inhibited polymerization reactions. It is also noteworthy that from day 13 onwards oil samples started to acquire a brownish color, probably due to Maillard-type browning reactions. During the storage of sunflower oil samples enriched with 10% GEO this band near 3469 cm^{-1} associated with the overtone of the carbonyl group absorption of triglyceride esters, underwent a completely different evolution in comparison with that of non-enriched oil and oils enriched with 0.005% and 0.5% GEO. This band also widened, although to a lesser extent (see Fig. 2d), but its frequency values barely varied over time (see Fig. 3a). Therefore, during the storage of this sunflower oil samples enriched with 10% GEO the three periods of the oxidation process discussed earlier (FP, SP and TP), could not be distinguished. The evolution of this band suggested that either hydroperoxides were not generated, or that they were produced in so low amounts that were not detectable by FTIR. In addition, the above-mentioned band near 987.5 cm^{-1} related to bond vibrations of the *cis,trans*- and *trans,trans*-conjugated olefinic groups CH of hydroperoxides was barely observed (see Fig. 2d). To confirm this hypothesis, all enriched and non-enriched sunflower oil samples submitted to accelerated storage conditions were also analyzed using ^{1}H NMR, as in previous studies (Goicoechea & Guillen, 2010). Fig. 4a shows some expanded regions of the ^{1}H NMR spectra of non-enriched sunflower oil and of sunflower oil enriched with 10% GEO after being submitted to accelerated storage for 0, 6 and 7 days. The assignment of the signals is provided in Table 3. These samples were selected because the study by FTIR evidenced that non-enriched oil contained the maximum concentration of hydroperoxides at day 7, as previously described. In the expanded regions of ^{1}H NMR spectrum of non-enriched oil that at day 0, it can be observed that before storage there were no signals related to the protons of primary or secondary oxidation compounds. However, at day 6, the presence of primary oxidation

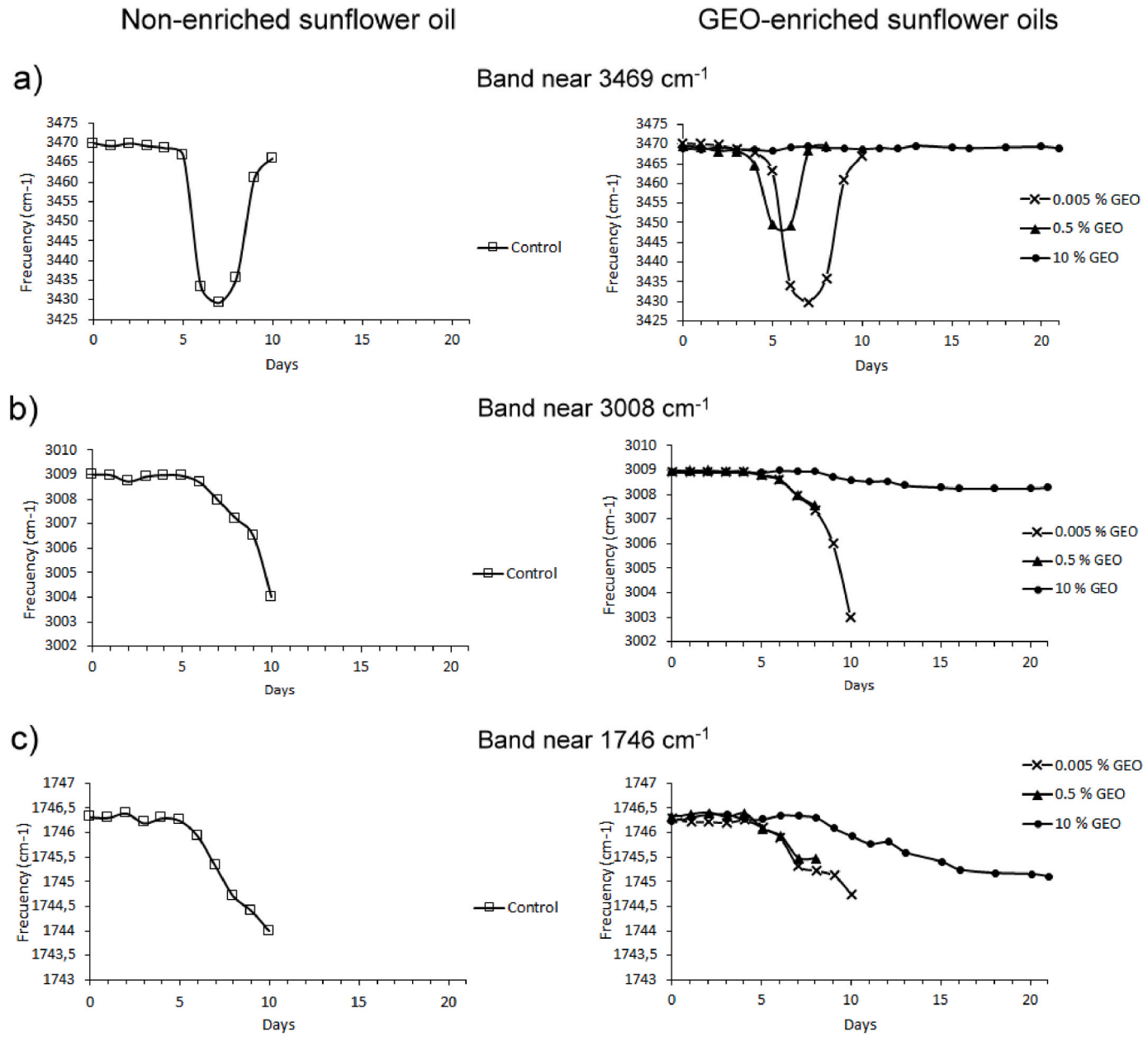

Fig. 3. Frequency values of the FTIR bands near **a)** 3469 cm^{-1}, **b)** 3008 cm^{-1} and **c)** 1746 cm^{-1} of non-enriched sunflower oil and sunflower oil enriched with GEO at 0.005 %, 0.5 % and 10 % of GEO submitted to accelerated storage.

compounds having *cis,trans*- (signal **k**) and *trans,trans*- (signal **l**) conjugated dienic systems supported on chains having also hydroperoxyl groups (signal **m**) was observed. Furthermore, incipient signals of aldehydic protons of secondary oxidation products were detected: signal **n** due to *trans*-2-alkenals, and signal **r** due to 4-hydroperoxy-*trans*-2-alkenals. As shown in the spectrum of non-enriched oil at day 7, as storage advanced, more signals related to aldehydic protons were observed in addition to signals **n** and **r**, this is: signal **o** of *trans, trans*-2,4-alkadienals, signal **p** of 4,5-epoxy-*trans*-2-alkenals, signal **q** of 4-hydroxy-*trans*-2-alkenals and signal **s** of alkanals. On the contrary, in the spectra of sunflower oil enriched with 10% GEO after being submitted to accelerated storage for 6 and 7 days, the above-described signals related to primary oxidation compounds were scarcely detected (signals **k**, **l** and **m**) and regarding secondary oxidation compounds, only incipient signal **s** related to alkanals was observed. These results confirmed that the amount of hydroperoxides generated in samples enriched with 10% of GEO was much lower than in the other samples, which is consistent with the evolution of the FTIR band near 3469 cm^{-1}. It must be noted that, as can be observed in Fig. 4a, signal **j** related to olefinic protons of allyl-sulfides present in GEO, decreased its intensity as storage advanced, as expected.

In summary, the analysis of the changes occurring in the FTIR band near 3469 cm^{-1} of the enriched and non-enriched oils spectra provided very valuable information on the oxidative stability and chemical transformations occurring in the oils under accelerated storage conditions.

3.2.2. FTIR band near 3008 cm^{-1}

3.2.2.1. Changes in the spectra of non-enriched sunflower oil during storage. As the storage of non-enriched sunflower oil samples advanced, some changes were also observed in the FTIR band located near 3008

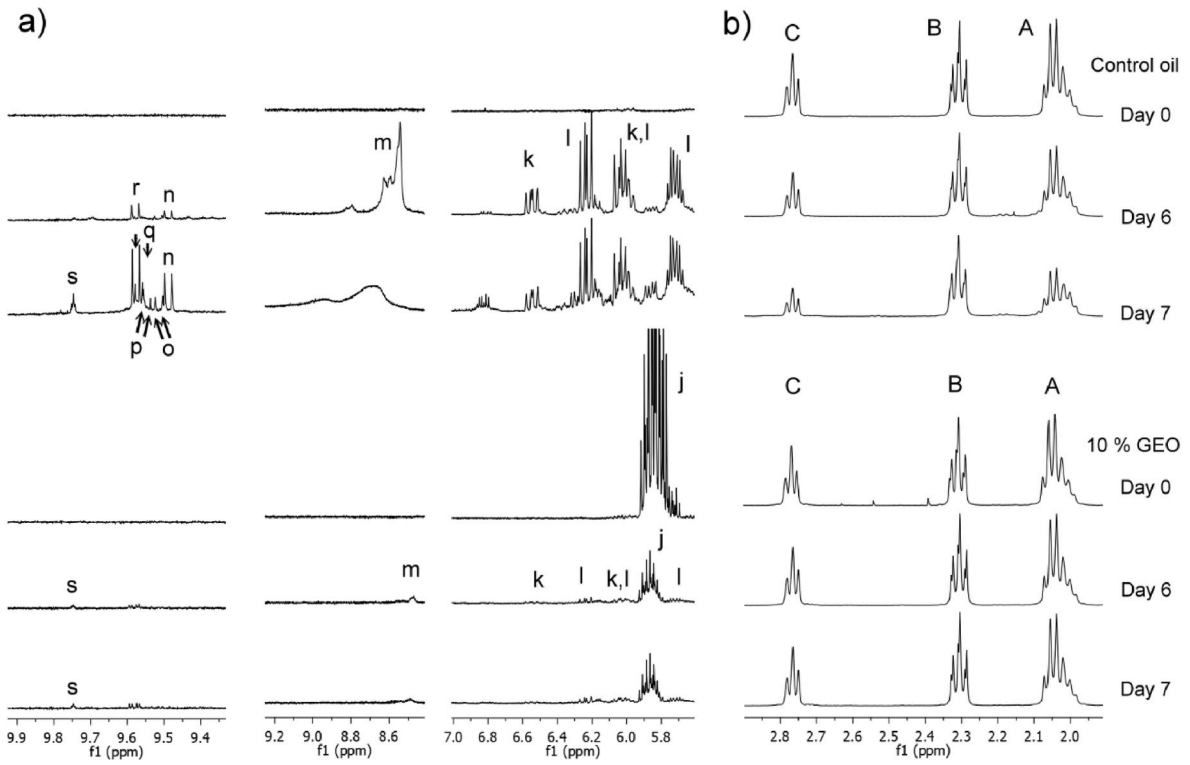

Fig. 4. Expanded regions of the ^{1}H NMR spectra of non-enriched sunflower oil and of sunflower oil enriched with 10 % GEO after being submitted to accelerated storage for 0, 6 and 7 days: **a)** regions at 5.7–7.0 ppm, 8.5–9.2 ppm and 9.3–9.9 ppm, where signals related to oxidation compounds appear; **b)** region at 1.9–2.9 ppm, where signals due to some protons of main acyl groups can be observed. Signal letters agree with those of Tables 2 and 3.

cm^{-1}, which is associated with the stretching vibration of CH in *cis* double bonds (Guillén & Cabo, 1997, 1999; Van de Voort et al., 1994). The frequency of this band is known to be related to the sample composition, in such a way that oils with high proportions of polyunsaturated acyl groups (linoleic or linolenic) show slightly higher values than those rich in monounsaturated ones (oleic). During the FP of the non-enriched sunflower oil, the frequency of this band remained almost unaltered (see Fig. 3b, days 0–5). At day 6 this frequency value started to decrease sharply, due to the disappearance of *cis* double bonds supported on unsaturated acyl chains, because of cleavage to generate primary and secondary oxidation products and/or isomerization to *trans* double bonds. It must be remembered that in this same spectrum of non-enriched oil at day 6, the changes observed in the intensity and frequency value of the band at 3469 cm^{-1} evidenced the formation of hydroperoxides, derived from the oxidation of unsaturated linoleic chains. Therefore, the period during which the frequency of this band near 3008 cm^{-1} remained unaltered can also be considered as a measure of the oxidative stability of the sample. Moreover, the lower the frequency value of this band, the more advanced is the oxidation process. At day 10 of storage, this band reached a minimum value of 3004.00 cm^{-1}.

3.2.2.2. Changes in the spectra of sunflower oils enriched with GEO during storage. In sunflower oil samples **enriched with 0.005% GEO**, the frequency of this band followed a similar evolution to that of the non-enriched oil. During the FP, it remained almost unchanged (see Fig. 3b, days 0–5). After this period, there was a clear and pronounced diminution in the frequency value of this band, due to a reduction in *cis* double bonds, reaching at day 10 a minimum value of 3003.12 cm^{-1}.

Regarding oil samples **enriched with 0.5% GEO**, the evolution of the frequency of this band near 3008 cm^{-1} was somewhat different from that of non-enriched oil, and from that of oil enriched with 0.005% GEO. During the FP, its frequency remained almost unchanged (see Fig. 3b until day 4). Afterwards, there was a slight decrease in the frequency values of this band, reaching a minimum frequency value of 3007.54 cm^{-1}, at the last day of storage (day 8). It must be noted that this minimum value is higher than those reached during the storage of non-enriched and 0.005% GEO-enriched sunflower oils. This indicated that during storage of sunflower oil enriched with 0.5% GEO a lower degradation of *cis* double bonds occurred.

On the other hand, in sunflower oil samples **enriched with 10% GEO** the frequency of this band hardly changed during accelerated storage (see Fig. 3b). This indicated that there has been a scarce degradation of the above-mentioned *cis* double bonds of linoleic acyl groups to generate primary and secondary oxidation compounds. These results were confirmed by ^{1}H NMR. As it can be observed in Fig. 4b, in the ^{1}H NMR spectrum of 10% GEO-enriched oil after being submitted to storage for 7 days, the signals of monoallylic (signal **A**) and bisallylic (signal **C**) protons remained almost unaltered; on the contrary, in non-enriched oil submitted also for 7 days to the same conditions, the intensity of both signals was much lower than in the spectrum of day 0, evidencing the degradation of unsaturated acyl groups.

3.2.3. FTIR band near 1746 cm^{-1}

3.2.3.1. Changes in the spectra of non-enriched sunflower oil during storage. As can be observed in Fig. 2, in the FTIR spectra of all oil samples a very intense absorption due to the stretching vibration of the ester carbonyl –C=O group of triglycerides was observed at 1746 cm^{-1} (Guillén & Cabo, 1997; 1999; Van de Voort et al., 1994). In non-enriched sunflower oil submitted to storage conditions, the frequency value of this band remained practically constant during the FP (see Fig. 3c, days 0–5). Afterwards, in the SP, its frequency began to decrease, reaching values close to 1744.00 cm^{-1} at the end of the TP (day 10). These changes can be attributed to the generation of aldehydes among secondary oxidation compounds, that cause an absorption at 1728 cm^{-1}, which overlaps with the band of the ester functional group (Van de Voort et al., 1994). It can be stated that those samples with frequency

Table 3
Chemical shift assignments and multiplicities of some ^{1}H NMR signals in $CDCl_3$ of the main acyl groups present in sunflower oil, and of the lipid oxidation products generated during accelerated storage conditions (Goicoechea & Guillen, 2010; Martínez-Yusta et al., 2014). The signal letters agree with those given in Fig. 4.

Signal	Chemical shift (ppm)	Multiplicity	Functional group	
Main acyl groups in triglycerides				
A	1.941–2.139	m[a]	-C**H**$_2$-CH=CH-	Unsaturated acyl groups
B	2.305	dt	-OCO-C**H**$_2$-	All acyl groups
C	2.765	t	= HC-C**H**$_2$-CH =	Linoleic acyl groups
Primary oxidation compounds				
k	6.58	dddd	-C**H**=C**H**-C**H**=C**H**-	*cis,trans*-conjugated double bonds associated with hydroperoxides (OOH)
k	6.00	dddt		
	5.56	ddm		
	5.51	dtm		
l	6.27	ddm	-C**H**=C**H**-C**H**=C**H**-	*trans,trans*-conjugated double bonds associated with hydroperoxides (OOH)
l	6.06	dddt		
l	5.76	dtm		
	5.47	ddm		
m	8.3–8.9	–	-OO**H**	hydroperoxide group
Secondary oxidation compounds: Aldehydes				
n	9.49	d	-C**H**O	*trans*-2-alkenals
o	9.52	d	-C**H**O	*trans,trans*-2,4-alkadienals
p	9.55	d	-C**H**O	4,5-epoxy-*trans*-2-alkenals
q	9.57	d	-C**H**O	4-hydroxy-*trans*-2-alkenals
r	9.58	d	-C**H**O	4-hydroperoxy-*trans*-2-alkenals
s	9.75	t	-C**H**O	alkanals

[a] overlapping of multiplets of the α-methylenic protons in relation to a single double bond of the different unsaturated acyl groups. Abbreviations: t, triplet; m, multiplet; d, doublet; s, singlet.

values of this band lower than 1746 cm^{-1}, a typical value in non-oxidized samples, are oils in which the oxidative process has begun; the lower the frequency value of this band, the more advanced the oxidation process in the sample is. Furthermore, the faster the frequency of this band starts to decrease, the lower the oxidative stability of the sample is (Guillén & Cabo, 1999).

3.2.3.2. Changes in the spectra of sunflower oils enriched with GEO during storage. In the FTIR spectra of sunflower oil **enriched with 0.005% GEO**, the frequency of this band near 1746 cm^{-1} also remained practically constant during the FP (days 0–5) and in the SP it began slightly to decrease. At the end of the TP it reached a minimum value of 1744.80 cm^{-1} (day 10), similarly to that occurring in non-enriched oil.

In the spectra of the oil **enriched with 0.5% GEO**, the frequency of this band remained practically constant during the FP (see Fig. 3c, days 0–4), and then in the SP, it began to decrease, reaching minimum value of 1745.50 cm^{-1} at the end of the TP (day 8). This value is higher than the minimum frequency value of the same band in the spectra of non-enriched and 0.005% GEO enriched oils, which can be attributed to a lower generation of aldehydes in the former than in the latters.

Regarding the oil **enriched with 10% GEO**, the frequency of this band also decreased, but at a slower rate than in the other enriched and non-enriched oils. Until day 9 it remained almost unaltered, and from day 10 onwards it became lower than 1746 cm^{-1}, reaching a minimum frequency value of 1745.13 cm^{-1} after 21 days of accelerated storage. This value is slightly higher than in the other enriched and non-enriched oils. These results evidenced a lower and delayed generation of aldehydes in 10% GEO enriched oil, which is in agreement with that above-described regarding ^{1}H NMR results (see Fig. 4a).

On the whole, the results obtained evidenced that the effect of GEO on sunflower oil oxidative stability depended on the concentration added, in such a way that the addition of GEO at 0.005% did not affect the oxidative stability of sunflower oil. These results are in agreement with those reported by Sun et al. (2000), who found that the addition of GEO at 0.006% to Chinese-style heat-dried sausages did not provide any significant antioxidant effect, as measured by the Thiobarbituric acid test (TBA) test. Sallam et al. (2004) studied the antioxidant activity of different garlic formulations added to raw chicken sausages during refrigerated storage using Peroxide Value and TBA test, and they reported the lowest antioxidant activity for garlic oil (0.006%, 0.009% and 0.015%), compared to the other garlic formulations (fresh garlic and garlic powder).

Conversely, in this study, a 0.5% GEO enrichment level provoked a decrease in sunflower oil oxidative stability during storage, as the generation of hydroperoxides was detected one day earlier than in non-enriched oil (day 4 vs. day 5), albeit in lower concentrations in the former than in the latter oil samples. Additionally, during the storage of 0.5% GEO-enriched oil, lower concentrations of aldehydes were generated, less degradation of *cis*-double bonds of unsaturated acyl groups was observed, and the oil polymerized two days earlier (day 8 vs. day 10) compared to non-enriched oil. These results are in line with those provided by Nieto et al. (2012), who reported that GEO added at 0.4% to pork burgers exerted a prooxidant effect, studied by Electron Spin Resonance.

The addition of 10% GEO increased oil oxidative stability, causing remarkable differences in the oxidation process: hydroperoxides were scarcely generated, lower concentrations of aldehydes were detected, the *cis*-double bonds of unsaturated acyl groups were degraded in much lower proportions, and much more time was needed for polymerization reactions to occur. In view of the results obtained, small amounts of this 10% GEO-enriched sunflower oil could be used as an ingredient in food products, to flavor them with garlic aroma and increase their oxidative stability. If flavoring is not intended, encapsulation techniques could be used to preserve GEO activity and minimize its effect on sensory quality (Tsitlakidou et al., 2023).

4. Conclusions

This study on the effect of GEO on sunflower oil oxidative stability during accelerated storage conditions using FTIR spectroscopy has demonstrated the usefulness of this technique for monitoring the changes occurring in lipids under these oxidative conditions, in a fast a simple way (1–2 min), and without any previous sample modification, which in turn avoids the formation of artifacts. As described above, it was possible to obtain very valuable information on the oxidation processes by paying attention only to three bands of the FTIR spectra.

Considering the results obtained, it can be concluded that the effect of any potential antioxidant compound or mixture of compounds must be studied carefully, because it can differ depending on the concentrations tested. In this context, it is of great importance to use specific methodologies, such as FTIR, which able to track the degradation of substrates (unsaturated lipids) and the formation of oxidation compounds, and to conduct the study under the oxidative conditions as close as possible to those of the real system in which it would be applied.

CRediT authorship contribution statement

Izaskun Martín-Cabrejas: Writing – original draft, Investigation. **Encarnacion Goicoechea-Oses:** Writing – review & editing, Writing – original draft, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Data availability

Data will be made available on request.

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