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Spectroscopic Characterization of Ozonated Sunflower Oil.

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Abstract

Ozonation reactions are very important in vegetable oil chemistry since their ozonation products are involved in antimicrobial effect in therapeutical uses for several microbiological etiology diseases. Information on the spectroscopic characterization of the products generated by ozonolysis of sunflower oil is limited. In the present study ozonized sunflower oil with 650 mmol-equiv/kg of peroxide index is chemically characterized. Ozonation of sunflower oil produced ozonides, aldehydes and hydroperoxides which were identified by ^{1}H, ^{13}C and two-dimensional ^{1}H Nuclear Magnetic Resonance (NMR). The virgin sunflower oil and ozonized sunflower oil show very similar ^{1}H NMR spectra except for the resonances at $\delta = 9.74$ and $\delta = 9.63$ ppm that correspond to both triplet from aldehydic protons, $\delta = 5.6$ ppm (olefinic signal from hydroperoxides), and $\delta = 5.15$ ppm (multiplet from ozonides methylic protons). Other resonance assignments are based on the connectivities provided by the proton scalar coupling constants. These are the following: $\delta = 3.15$ ppm (doublet from methylenic group in α position respect to olefinic proton), $\delta = 2.45$ ppm (multiplet from methylenic group allylic to ozonides methynic protons) and $\delta = 1.62$ ppm (multiplet methylenic protons in β position respect to ozonides methynic protons). From the ^{13}C NMR and ^{1}H-^{13}C two- dimensional spectrum of the ozonized sunflower oil, the presence of ozonides was confirmed by the signals $\delta = 103.43$ and $\delta = 103.49$ ppm, respectively. The others new signals found in $\delta = 42.5$ and $\delta = 42.76$ ppm confirm the presence of methylenic carbons from hydroperoxides and ozonides. These results indicate that NMR Spectroscopy can provide valuable information about the amount of reaction compounds of ozonized vegetable oil. From the chemical structural elucidation of ozonated sunflower oils, relevant biochemical and chemical information can be achieved.

Key Words

Ozone; NMR; Sunflower Oil; Ozonides; Ozonated Sunflower Oil;

Introduction

The vegetable oils are formed in 97-98% by triglycerides. Depend on their origin and nature they have a variable composition of saturated and unsaturated fatty acids, for example, the oleic, stearic, palmitic, linoleic, lauric, mirystic and linolenic acid, all them are bonded to the glycerol backbone (Firestone, 1999). In the ozonized vegetable oils the obtained composition is even more complex that of any virgin vegetable oils (Díaz et al., 2001), (Ledea et al., 2001). The interaction of the ozone with unsaturated molecules from vegetable oils generates the formation of a mixture of chemical compounds such as ozonides, peroxides and aldehydes (Bailey, 1978). The ozonides and peroxides have high germicide effect of great utility in the field of medicine (Díaz et al., 2001).

The sunflower oil is normally renowned for its high linoleic acid and oleic acid content (Harris et al., 1978) Ozonized sunflower oil OLEOZON® is a wide spectrum antimicrobial product with inhibitory and lethal activity on grampositive and gramnegative bacterias, resistant strains to the antibiotics, *mycobacterium* species, yeasts of the gender *Candida* as well as some protozoos like the *Giardia lamblia* (Lezcano et al., 1996), (Lezcano et al., 1998) (Menéndez et al., 1995). This product has approved the Medical Registry for the Epidermofitosis disease, pathology of fungicide origin very frequent in many countries (Menéndez et al., 1999).

Information about the compounds generated by ozonolysis of sunflower oil is limited. Nuclear Magnetic Resonance spectroscopy has played and ever-increasing role in the study of properties of oils of vegetable origin during the last years. High-resolution ^{1}H NMR spectroscopy has had limited use in vegetable oil analysis owing to the small range of chemical shifts covered by protons, which resulted in the small number of signals in the proton spectrum. ^{13}C spectra of vegetable oil provided a large number of signals spread over a wide range of chemical shifts, which made the spectrum appear complicated but much more informative. Spectra DEPT (Distortionless Enhancement by Polarization Transfer. Techniques of correlating signals between ^{1}H and ^{1}H and between ^{1}H, and ^{13}C NMR spectrum provided two-dimensional correlation spectrum (2D COSY), which allowed confirmation of signals (Soon, 1984), (Marcel et al., 1997), (Giovanna, 1998), (Vlahov, 1999), (Díaz et al., 2003). Our aim in this study is to apply ^{1}H, ^{13}C and 2D COSY NMR spectroscopy, as analytical tool for chemical characterization of ozonized sunflower oil.

Materials and Methods

Materials

The edible quality sunflower oil was supplied by Ecasol S.A. Argentina. All other reagents used were of analytical grade.

General ozonation procedure

Sunflower oil (150 g) was introduced into a reactor with bubbling ozone gas and placed in water bath at room temperature. The reaction with ozone was continued for 2 hours. Ozonized sunflower oil was stored between 2-8 ^{0}C before further analysis for NMR.

Generation of ozone

Ozone was generated by passing oxygen through a Trailigaz Labo model 12-02 ozone generator at a fixed voltage (170 V) and a constant flow rate of 42 L/h. Resulting ozone concentration was 79,5 mg/L determined by an Anseros Ozomat equipment.

Measurement of ^{1}H-NMR spectra

^{1}H-, ^{13}C-, DEPT and 2D COSY NMR spectra were obtained with a Bruker 400 MHz and 100 MHz AVANCE Spectrometer with $CDCl_3$ as solvent, tetramethylsilane (TMS) as internal reference.

Determinations

Peroxide index

The peroxide index represents the number of mmol-equivalents of active oxygen that expresses the amount of peroxide contained in 1 000 g of the substance. Briefly, 5 g of sample was mixed with 30 volumes of glacial acetic, 20 volumes of chloroform and 0.5 mL of saturated potassium iodide solution. The mixture was shaken for exactly 1 minute, mixed with 30 mL of water and slowly titrated, shaking continuously, with 0.01 M sodium thiosulphate until the yellow color almost disappears.

The peroxide index values were obtained from the expression 10v/w, where v is the volume of sodium thiosulphate in mL consumed in the titration, and w is the weight in g of substance (BP, 1998). The peroxide index (PI) was expressed in mmol-equiv/kg.

Results and Discussion

Sunflower oils is a mixture of triacylglycerols (triglycerides) that contains unsaturated fatty acid such as oleic acid and linoleic acid, the contents of which are known to be (14-39.4 %)

and 48.3-74.0 %, respectively (Codex, 1993). In our experiment for preparation of ozonated sunflower oil, about 61.2 mg of ozone was absorbed per 1.0 g of sunflower oil which seemed to be enough to obtain ozonized sunflower oil with 650 mmol-equiv/kg of peroxide index.

Figure 1 shows ^{1}H NMR spectrum from virgin sunflower oil, presenting a single peak at δ = 7.26 ppm which below to the chloroform-d, multiplet peaks at δ = 5.35 ppm which belong to olefinic signals from the fatty acid and, δ = 5.20 ppm (triplet from methynic group of glycerol in *sn*-2 position). Other present signals are δ = 4.14 to δ = 4.29 ppm (doublets belonging to glycerol protons in *sn* 1,3 position); δ = 2.76 ppm (triplet from methylenic group between olefinic protons); δ = 2.30 ppm (triplet from methylenic groups in α position with respect to carbonylic group); δ = 2.03 ppm (multiplet from methylenic group in both sides of olefinic protons); δ = 1.60 ppm (multiplet from methylenic group in β position with respect to carbonylic group); δ = 1.30 ppm (signal from methylenic groups in fatty acid chain); and δ = 0.88 ppm (triplet from terminal methyl group).

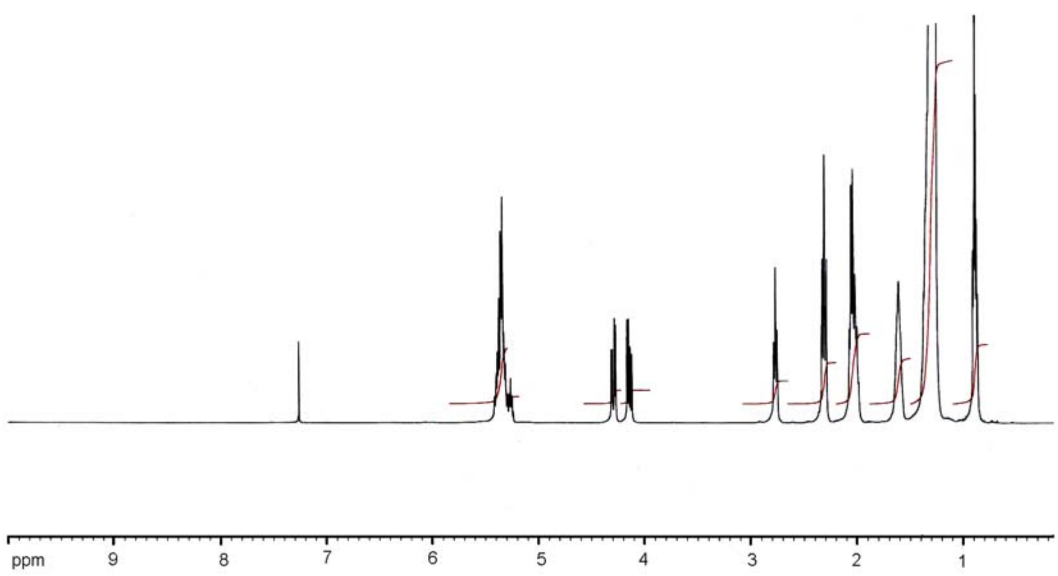

Figure 1. 400 MHz ^{1}H NMR spectrum of virgin sunflower oil.

^{1}H NMR spectrum from ozonated sunflower oil is displayed in figure 2. This spectrum has the same observed signals in virgin sunflower oil (figure 1) and additionally other eight signals at δ = 9.74 ppm y δ = 9.63 ppm (triplets from aldehydic protons), δ = 5.6 ppm (olefinic protons signal could be come from hydroperoxides), δ = 5.15 ppm (multiplet from ozonides), δ = 3.15 ppm (doublets from methylenic protons alilic of olefinic protons), δ = 2.45 ppm, δ = 1.67 ppm, and δ = 1.32 ppm (multiplet from formed ozonides protons). These formed additionally signals are oxygen compounds responsible for the germicide effect of ozonized sunflower oil (Díaz et al., 2001).

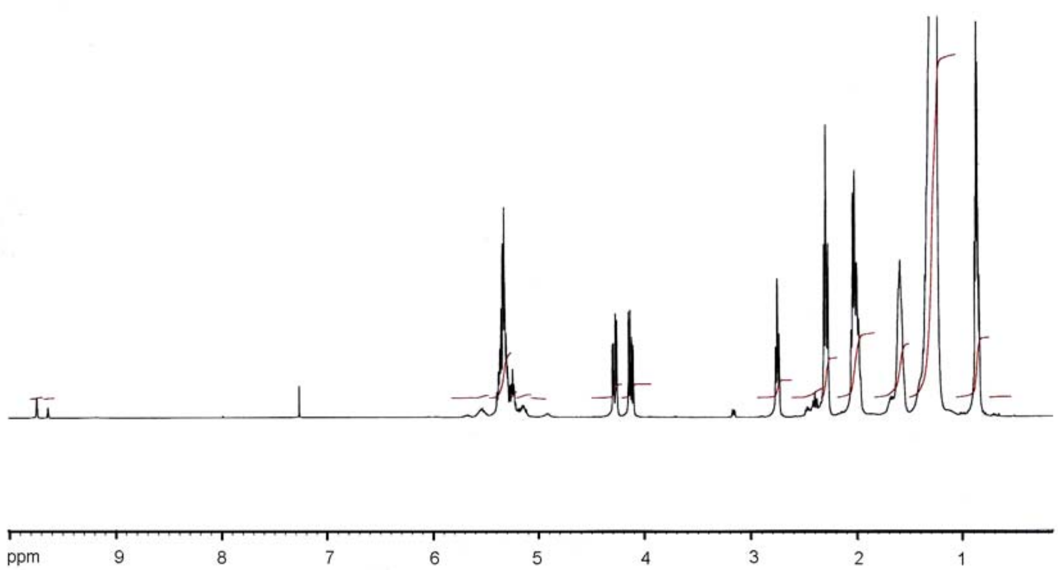

Figure 2. 400 MHz ^{1}H NMR spectrum of ozonated sunflower oil.

The ^{13}C NMR spectrum of a vegetable oil sample contains the resonance of carbons from the triglyceride fraction of vegetable oil, i.e. the fatty acid resonance. The carbon-13 resonance are grouped in four sets of signals, carbonyl carbons resonating from 171 to 174 ppm, unsaturated carbons in the range from 124 to 134 ppm, glycerol backbone carbons from 60 to 72 ppm and aliphatic carbons from 10 to 35 ppm.

Spectral region 14-35 ppm:

Figure 3 shows ^{13}C and DEPT NMR spectra from virgin sunflower oil. The aliphatic carbons of methyl and methylenic groups resonate in the range of 14-34 ppm. The different signals are resolved on the basis of chain double bond numbers: saturated (n=16-22:0), oleoyl (C18:1 9c) and linoleoyl chains (C18:2 9,12c) resonate from high to low frequency in that order. The chemical shift can be predicted by the additive relationshift for the normal alkanes based upon the number of α, β and γ carbon atoms in the molecule (Giovanna, 1998). The terminal methyl carbon shift of different chains C-18 is found at δ = 14.03 and δ = 14.06 ppm, respectively. Four methylene groups are readily identified: C-17 at δ = 22.54 and δ = 22.65 ppm methylenic acylic chains; C-3 at δ = 24.81 ppm methylenic group in β position with respect to carbonylic group; C-11 at δ = 25.59 ppm methylenic group between olefinic protons of linoleyl chains; C-11(0); C-14(L); C-8(O) C-8(L) at δ = 27.16 ppm allylic carbons of oleoyl and linoleoyl chains; δ = 29.08-29.73 ppm methylenic groups in fatty acid central chain; C-16 at δ = 31.49 methylenic acylic chains ω; C-2 at δ = 33.98 oleoyl and linoleoyl as a single signal but well resolved from saturated chains, could be differentiated according to 1,3- and 2-position on glycerol backbone.

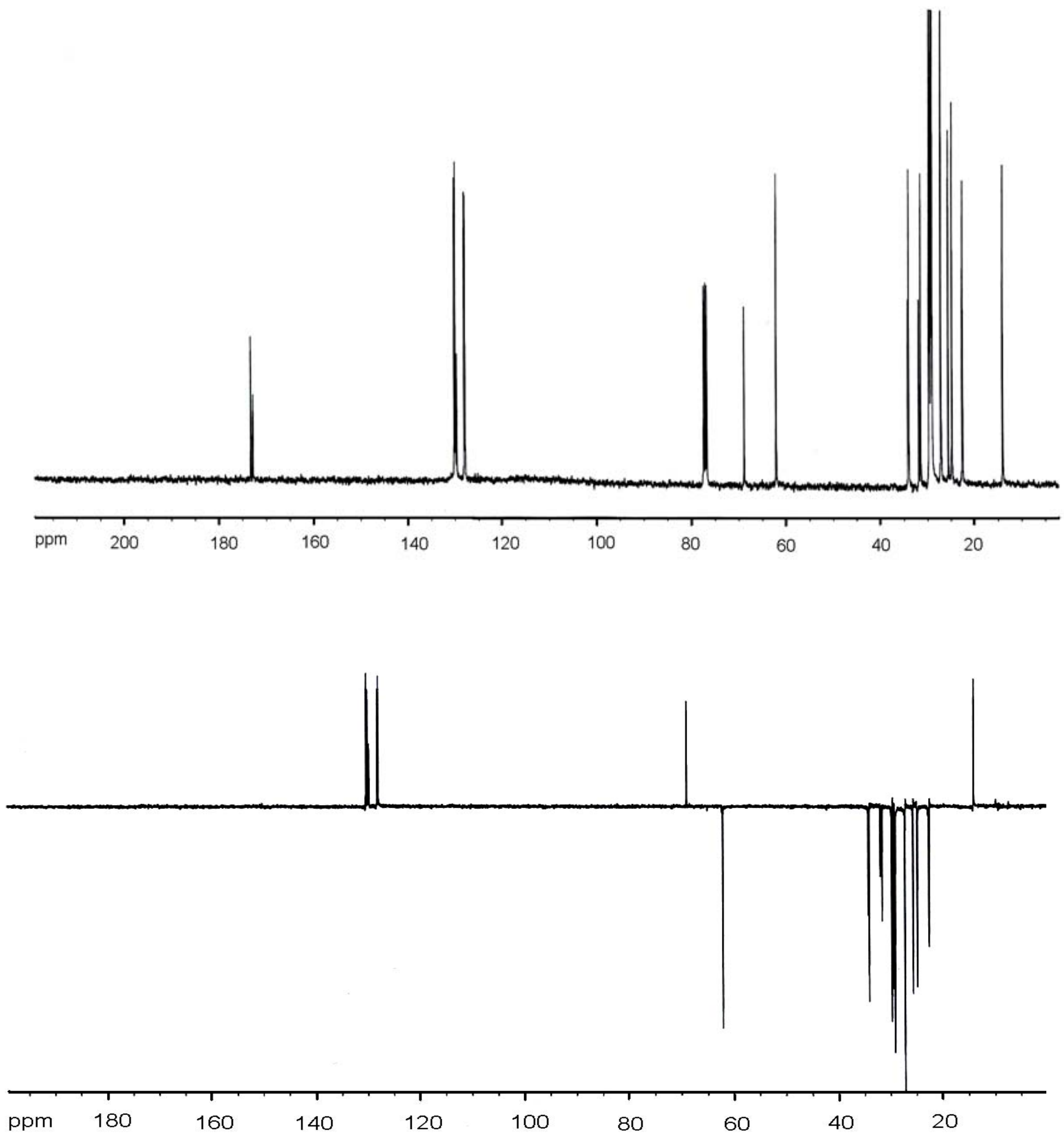

Figure 3. 400 MHz ^{13}C and DEPT NMR spectra of virgin sunflower oil.

Spectral region 62-69 ppm:

The composition of mono-, di- and triglycerides of vegetable oils can be determined by means of the glycerol carbon resonance in the chemical shift range from 60 to 73 ppm (Vlahov, 1996). The 1(3)-and 2- glycerol carbons of triglycerides resonate at δ = 62.05 ppm and δ = 68.86 ppm, respectively.

Spectral region 127.0-132 ppm:

The resonance of unsaturated carbons of long chain acids of triglycerides spread over the chemical shift range from 127.0-132 ppm. The carbons C-12 and C-13 at δ = 127.86 ppm and δ = 128.03 ppm corresponding to linoleoyl unsaturated carbons between 2- and 1(3) positions of glycerol; C-9 and C-10 at δ = 129.65 ppm and δ = 130.14 ppm corresponding to oleoyl unsaturated carbons between 2- and 1(3) positions of glycerol. The resonance of unsaturated carbons of long chain acids of triglycerides spread over the chemical shift range from δ = 126.9 to 132 ppm.

Spectral region δ = 172-174 ppm:

The carbonyl carbons at δ = 172.73 ppm belongs to 2-glycerol chain positions and δ = 173.14 ppm belong to 1(3) glycerol chain positions. The carbonyl carbons of fatty acids from vegetable oil triglycerides appear as two sets of resonance, the high frequency set includes the chains esterified at 1(3)-glycerol positions, whereas the low frequency set includes the 2-glycerol chain positions according to the assignments based on synthetic models of triacylglycerols (Wollenberg, 1990).

The DEPT experiment was applied to obtain ^{13}C NMR spectra over the whole carbon-13 frequency range with the purpose of producing ^{13}C NMR resonance for more precise interpretation. The only drawback was the loss of carbonyl carbons resonance, which are no detected by the DEPT sequence (Freeman, 1998).

The ^{13}C NMR spectra of ozonated sunflower oil contains the same signals observed from ^{13}C NMR spectra of virgin sunflower oil (figure 3). However a new group of signals were found in the spectra from ozonated oil. These were grouped in seven sets of signals: aldehyde carbons resonating from 199 to 203 ppm, carbonyl carbons from 171 to 174 ppm, unsaturated carbons in the range from 124 to 134 ppm with the signals additional from 133 to 134 ppm, methynic carbons corresponding to ozonides and hydroperoxides from 104 to 122 ppm, glycerol backbone carbons from 60 to 72 ppm, methylenic carbons belong to ozonides and hydroperoxides from 42 to 44 ppm, and aliphatic carbons from 10 to 35 ppm with new signals from 23 to 24 ppm belong to methylenic carbons of ozonides.

The assignments of the various signals were accomplished by using a combination of 2D COSY techniques (^{1}H-^{1}H and ^{1}H-^{13}C) (Figure 5 and 6). To explain the detailed interpretation of the ^{1}H-^{1}H NMR COSY spectra, the ^{1}H NMR spectrum showed four new signals: δ = 5.6

ppm, δ = 5.15 ppm, δ = 3.15 ppm and δ = 2.45 ppm. From the ^{1}H-^{1}H NMR COSY correlation spectrum (Figure 5), it was clear that the multiplet at δ = 5.6 ppm (olefinic protons signal could belong to hydroperoxides) was correlated to the protons at δ = 3.15 ppm (doublets from methylenic protons alilic of olefinic protons); and δ = 5.15 ppm (multiplet from ozonides) was correlated to the δ = 2.45 ppm, δ = 1.67 and δ = 1.32 ppm (multiplet from formed ozonides protons).

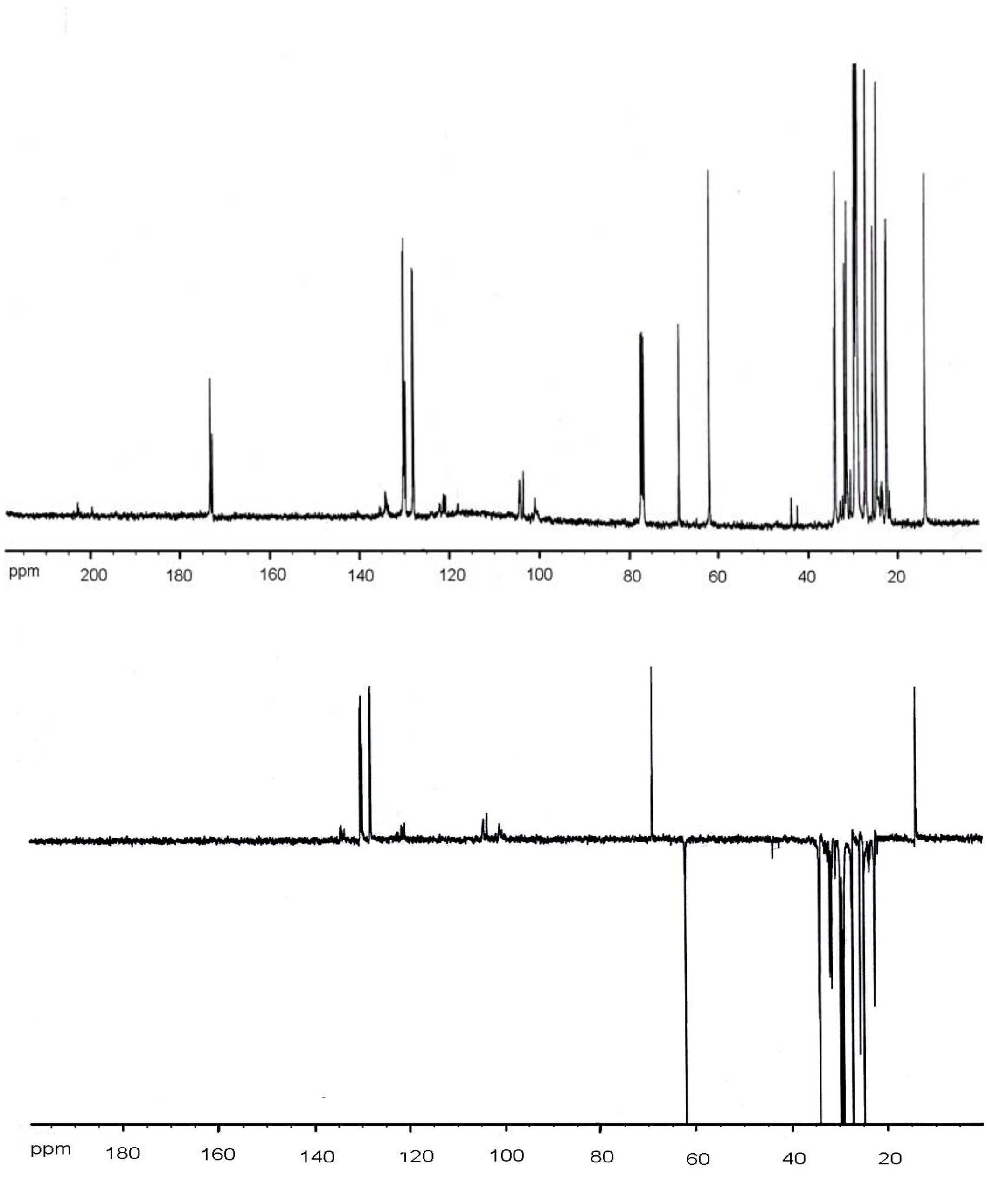

Figure 4. 400 MHz ^{13}C and DEPT NMR spectrum of ozonated sunflower oil.

The ^{13}C spectrum showed various new signals: δ = 133.77-134.2 ppm; δ = 121.18-121.98 ppm; δ = 104.26-103.51 ppm; δ = 43.84 ppm; δ = 42.55 ppm; δ = 23.45-23.88. From the ^{1}H-^{13}C NMR COSY correlation spectrum (Figure 6), we observed that the multiplet at δ_H = 5.6 was correlated to the carbon atoms δ_C = 133.77-134.2 ppm, spectral region of unsaturated carbons. The multiplets δ_H = 5.6 ppm and δ_H = 5.35 ppm were correlated to the carbon atoms δ_C = 121.18-121.98 ppm, spectral region of methynic carbons near of unsaturated carbons. The multiplet δ_H = 5.15 ppm was correlated to the carbon atoms δ_C = 104.26-103.51 ppm, they belong to carbons methynic from ozonides and oligomers, these assignations are similar to those reported by Miura (Miura, 2001) working with ozonated olive oil. Signal of methylenic carbon at δ_C = 43.84 ppm was correlated to the δ_H = 5.15 ppm of ozonides. Others signals of methylenic carbon at δ_C = 42.55 ppm was correlated to the δ_H = 5.6 ppm (olefinic protons signal could belong to hydroperoxides), and the last methylenic signals at δ_H = 1.32 ppm, belonging to ozonides, were correlated to the carbon atoms at δ_C = 23.45-23.88 ppm.

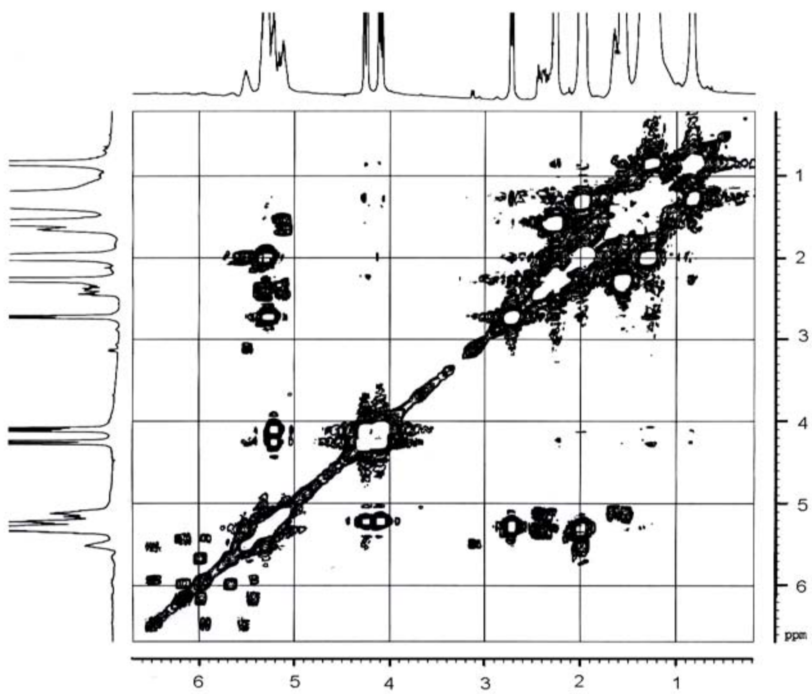

Figure 5. 400 MHz ^{1}H-^{1}H NMR correlation (COSY) spectrum of ozonated sunflower oil.

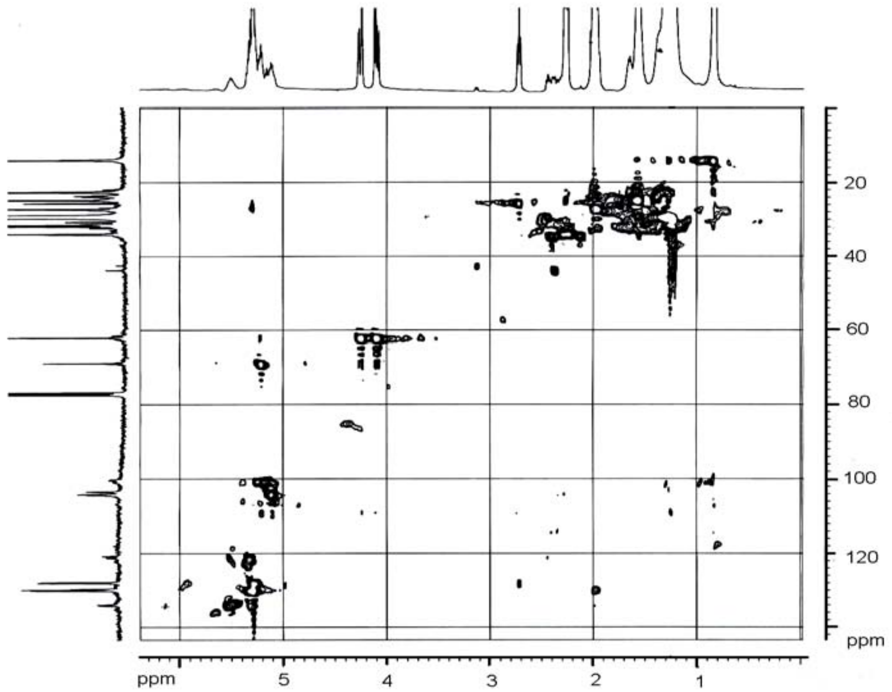

Figure 6. 400 MHz ^{1}H-^{13}C NMR correlation (COSY) spectrum of ozonated sunflower oil

In the structural elucidation of the ozonated sunflower oil, the assignments of various signals (ozonation products) were accomplished by using a combination of 2D COSY techniques (^{1}H-^{1}H and ^{1}H-^{13}C) with ^{1}H and ^{13}C NMR spectra. In this study the ozonation products were well characterized as ozonides, hydroperoxides and aldehydes present in ozonated sunflower oil. The ozonides have been considered one of the active principles of ozonated sunflower oil OLEOZON® (Díaz, et al., 2001). The elucidation and chemical characterization of active principles from ozonated sunflower oil are important for new ozonation strategies with this oil.

Acknowledgements

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