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Properties of sesame oil by detailed ^{1}H and ^{13}C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements

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ABSTRACT

Gaseous ozone chemically reacts with unsaturated triglyceride substrates leading to ozonated derivatives with a wide potential applications, ranging from the petrochemical to the pharmaceutical industry. To date, an ultimate understanding of the ozone reactivity during sesame oil ozonation process as well as detailed ^{1}H and ^{13}C NMR assignments are lacking. A practical advantage of NMR is that a single NMR sample measurement can explain many issues, while similar analysis by traditional methods may require several independent and time-consuming measurements. Moreover, significant relationships among NMR spectra and both conventional chemical analysis and viscosity measurements have been found. Eventually, NMR could play an important role for quality attributes of ozonated oil derivatives.

1. Introduction

Ozone's significant use in the petrochemical and pharmaceutical industry results from its reactivity as well as specific effects on the olefinic double bonds (Horváth et al., 1985; Soriano et al., 2003; Sadowska et al., 2008). Gaseous ozone chemically reacts with unsaturated substrates mostly leading to ozonated cyclic species, as schematically shown in Scheme 1. The postulated mechanism, known as the Criegee reaction, is based on the reaction that ozone works out on an unsaturated bond to form an initial, unstable primary ozonide. Such a primary ozonide readily decomposes to form a zwitterion and a carbonyl fragment which can combine to give either cyclic trioxolane compounds in anhydrous environments, or to a series of aldehydes and peroxides (Criegee, 1975). From a pharmaceutical point of view, the anti-infective value of the former has been increasingly appreciated during the last fifteen years. In fact, it has been realized that ozone, under various formulations, can display a cleansing effect and act as a potent disinfectant able to practically kill all pathogens present in the skin and mucosal surfaces. Furthermore, the decomposition of ozonated derivatives, by increasing the availability of oxygen in the ischemic or inflamed tissues, gives the additional advantage to improve the local metabolism and the proliferation of tissues, essential for the eventual mucosal or/and cutaneous healings (Travagli et al., 2009).

The vegetable oil ozonides are all liquid or semisolid at room temperature and have stability periods that may be adequate for commercial distribution and use purposes at usual storage conditions. However, the ozonide of the glyceryl esters are not very stable in the presence of light where the zwitterion may dimerizes to form a diperoxide derivative. Moisture could also facilitate the glyceryl oil-based ozonides break-down releasing the oxygen which also oxidizes the ozone-opened olefinic linkages, breaking the long chain Criegee biradical. Such a species is highly energized and can decompose along a number of different reaction pathways, among which the formation of aldehydes is prevalent (Pryor et al., 1991; Thornberry and Abbatt, 2004). As a result, rancidity develops creating objectionable odors.

From an industrial applicative viewpoint, the overall quality of ozonated derivatives depends upon several parameters, such as: the type and the quality of ozone generators; the efficacy of the ozonizer, in terms of ozone concentration output, gas carrier, gas flow; the ozonation conditions, in terms of reactors and time, material type and amount, presence of water and/or catalyzers.

The peroxide value is typically used as an indicator of the advancement and/or the control of the process because of its simplicity, rapidity and low cost, even if it had been necessary to standardize the methodology (Zanardi et al., 2008). Moreover, it is believed indispensable that each ozonated oil has to be unequivocally characterized in terms of the species contents as well as the

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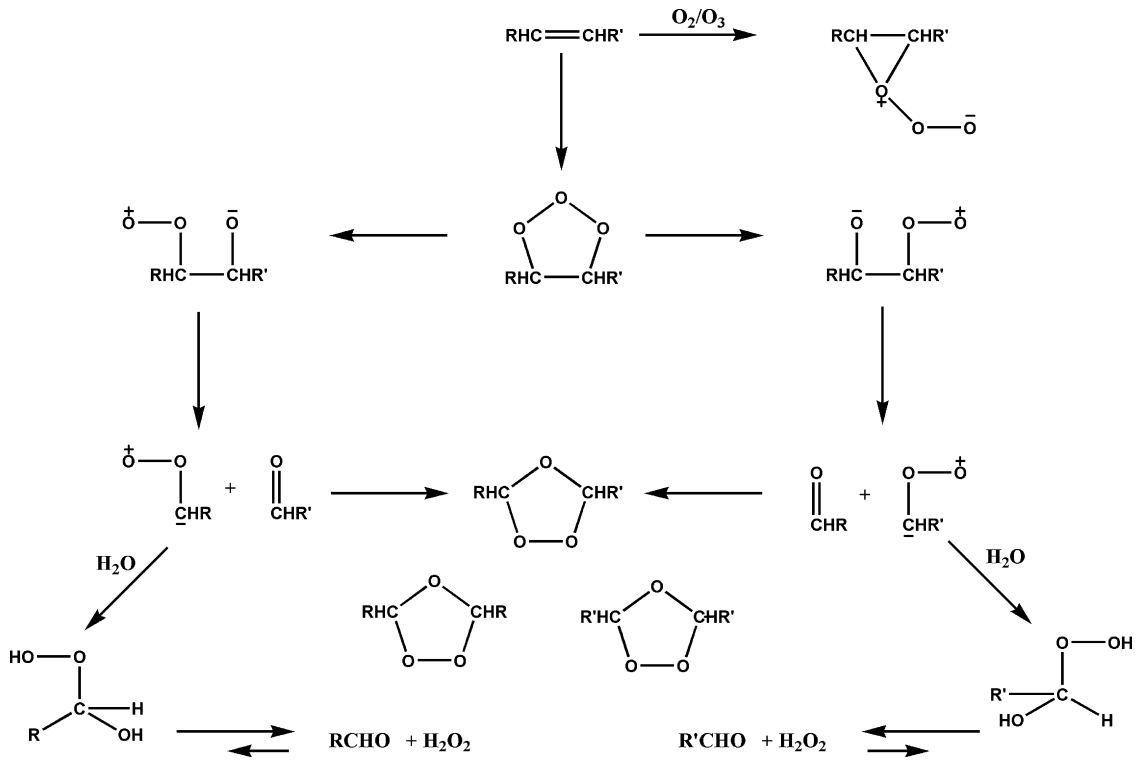

Scheme 1.

reaction kinetics. For this purpose, ^{1}H and ^{13}C NMR assignments of the characteristic moieties have been performed. The use of NMR for such purposes (Zhou et al., 2000; Giordano et al., 2007; Li et al., 2009) as well as for specific characterization of oils (Guillén and Goicoechea, 2007; Retief et al., 2009) and their ozonation products (Diaz et al., 2001; Soriano et al., 2003; Sadowska et al., 2008; Zanardi et al., 2008) is well established, even if for the latter some of the spectral details are not entirely assigned. In the present study, a comprehensive analysis based on NMR spectroscopic technique of pure sesame oil before and after ozonation has been performed. Sesame oil was selected for its wide use in pharmaceutical as well as for its chemical distribution of double bonds, with a balance between mono- and di-unsaturation. Such a knowledge allows an in-depth acquaintance of the ozonation process as well as of the related products obtained, allowing quality attribute understanding that appears useful for industrial purposes.

2. Experimental

2.1. Chemicals

Chemicals were purchased from Sigma–Aldrich and used without further purification. Sesame oil, hereinafter referred to as SO, matching the European Pharmacopoeia monograph (European Pharmacopoeia, 2008a) was purchased from Galeno Srl (Comeana, Italy).

2.2. Ozonation method

For clarity's sake the terms "ozonated" and "ozonation" will be used in the present paper, even if the synonyms "ozonized", "ozonizated", and "ozonization" could be found in the literature. The ozonated SO was produced by a method recently described (Zanardi et al., 2008). Briefly, ozone/oxygen mixture was bubbled in Drechsel bottles containing 40 mL of SO for different times comprised between 15 and 120 min (SO15, SO30, SO45, SO60, SO75, SO90, SO105, and SO120), corresponding to about 1012.5–8100 mg of ozone dose (see Table 1). The ozone flow-rate was kept constant at 1.5 L/min in all the experiments, and ozone concentration was 45 mg/L. The Drechsel bottle was weighed both before and after ozonation process in order to evaluate the weight increase.

2.3. NMR analysis

^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance 400 MHz spectrometer at 25 °C in $CDCl_3$. All the experiments were performed under the same experimental conditions and at same sample concentration (about 100 μL of oil sample in 750 μL $CDCl_3$). The ozone action on SO was studied by ^{1}H NMR at fixed time interval (namely 15′). ^{13}C NMR analysis was done on untreated SO and after 60′, 105′, and 120′ of ozone treatment. COSY and HMQC experiments were made on untreated SO and after 60′ and 120′ of ozone treatment. All ^{1}H NMR spectra were normalized using the integral areas of the OCH_2 protons (glycerol) that remains constant during the whole process.

2.4. Physico-chemical characterizations

Chemical characterizations (namely, iodine value, IV, and peroxide value, PV) of ozonated samples have been performed using

Table 1
Ozone dosage treatments for the different samples.

Sample	Ozone dose [mg]	Ozone dose [mg/mL of oil]
SO	0	0
SO15	1012.5	25.5
SO30	2025.0	50.6
SO45	3037.5	75.9
SO60	4050.0	101.3
SO75	5062.5	126.6
SO90	6075.0	151.9
SO105	7087.5	177.2
SO120	8100.0	202.5

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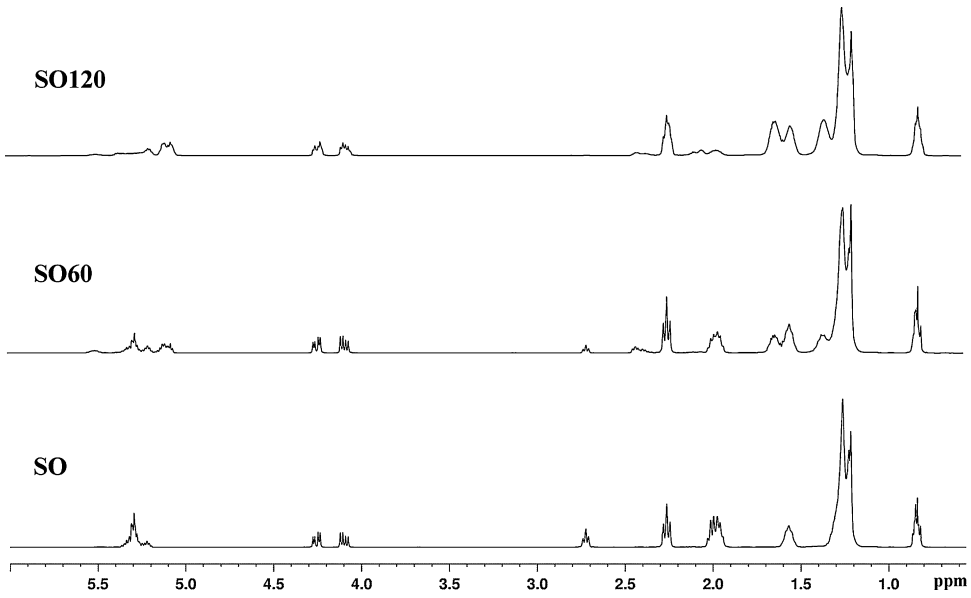

Fig. 1. ^{1}H NMR spectra of untreated SO and after 60′ (SO60) and 120′ (SO120) ozone treatment. Signals after 6.0 ppm are practically absent.

methods previously described (Zanardi et al., 2008). Particularly, PV was determined by means of both iodometric titration according with official monograph (European Pharmacopoeia, 2008b) as well as placing the sample at reflux for 60 min. Viscosity measurements have been obtained by torsional oscillation viscometer (Viscomate VM-10AL, CBC Europe) (Travagli et al., 2008) at both the temperatures of 22 and 35 ± 0.2 °C.

2.5. Ozonation efficiency

The ozonation efficiency (OE), expressed as a percentage, represents the ratio of the amount of peroxidation due to ozonation process, as estimated by PV value, to the amount of total ozone applied to the system. It was calculated by means of the following equation:

$$\mathrm{OE} = \frac{(\mathrm{PV_S} - \mathrm{PV_0})}{1000} \times \frac{\mathrm{O_3EW}}{\mathrm{AOD}} \times 100 \quad (1)$$

where $\mathrm{PV_S}$ is the ozonated sample peroxide value, $\mathrm{PV_0}$ is the SO peroxide value, $\mathrm{O_3EW}$ is the ozone equivalent weight and AOD represents the applied ozone dose (mg/g).

2.6. Statistical analysis

Results were expressed as the mean (CV% <2) of at least three independent measurements, unless standard deviations have been reported. The correlation graphs are obtained using Microcal™ Origin™ 5.0, and the correlation factor has been expressed by the r^2 parameter.

3. Results and discussion

3.1. NMR analysis

The complete NMR data of untreated SO are shown in Table 2. The main reaction is the formation of 1,2,4-trioxolane rings by ozone attack on the carbon–carbon double bonds as shown by signals at 5.11–5.08 (protons on ring carbons), at 1.63 (methylene protons α to ring carbons) and at 1.35 ppm (methylene protons β to ring carbons). These signals are steadily increasing during the ozonation process while the protons signals at 5.29 (CH=CH), at 2.72 (C=C–CH_2–C=C) and at 2.03–1.96 ppm (CH_2–C=C–CH_2 and CH_2–C=C–C–C=C–CH_2) are steadily decreasing (see Table 3 ; Fig. 1). The same behaviour can be followed in the ^{13}C NMR spectra (Fig. 2).

The trioxolane rings are very stable since proton signals from aldehyde groups are barely visible in the NMR spectra at any stage of ozonation process. The progress of ozone reaction is also accompanied by the appearance of weak signals at 5.50, 5.42–5.35, 5.35–5.28, 5.30–5.25, 2.42–2.35, and 2.12–2.04 ppm. Resonances in the interval ca. 5.4–5.2 are superimposed as can be seen in the HMQC spectrum (Fig. 3). Some of these resonances are present in the first control after 15′ of ozone reaction while others show up after 60′ (5.42–5.35, 5.35–5.28, and 2.12–2.04 ppm). Also the behaviour of these weak signals is different: some increase with time while others increase up to 75′ of ozone reaction and then decrease (5.50, 5.35–5.28, and 2.42–2.35 ppm) (Fig. 4). These signals are then associated with species that reacted further during the ozone treatment.

The protons resonances at 5.50 and 5.35–5.28 ppm are connected with carbons resonances in the HMQC spectra (Fig. 3) at 133–134 and 120–121 ppm, respectively. Thus, they belong to carbon–carbon double bonds and corresponding protons. These signals can be attributed to species deriving from reaction of ozone with one or the other of the two double bonds of the linoleic acid component of SO. Indeed, statistically most of linoleic moieties will react first at one of the double bonds and after at the second one. Such a consideration explains why these signals increase up to a certain time of ozone reaction and then decrease: the second double bond was under reaction. These facts also explain the behaviour of resonances at 2.42–2.35, and 2.12–2.04 ppm. The signals at 2.42–2.35 ppm belong to the methylene bridge protons connecting the 1,2,4-trioxolane ring to the double bond (see Tables 3 and 4), thus they will increase up to a certain time (75′) and then decrease on the attack on the other double bond by ozone. Indeed, the chemical shifts of the methylene bridge protons now connecting two rings are at 2.12–2.04 ppm and their signals are discernible in the NMR spectrum only after 60′ of ozone treatment. The resonances at 5.42–5.35, and 5.30–5.25 ppm related by HMQC to carbon resonances at 100.2 and 100.4 ppm also appear after 60′ of ozone treatment and can be assigned to carbon rings connected by a methylene bridge (see Tables 2 and 3, Fig. 3). Thus, all these resonances are related to the ozone reaction with the linoleic moieties of SO.

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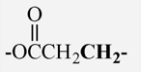
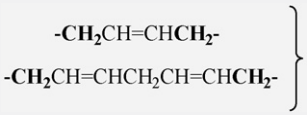
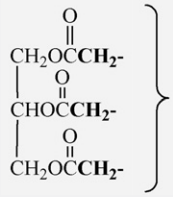
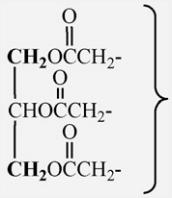
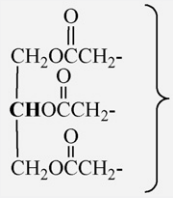
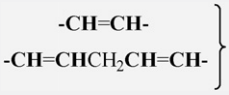
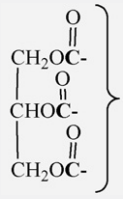
Table 2
^{1}H and ^{13}C NMR chemical shifts of untreated SO.

Moiety	^{1}H δ (ppm)	^{13}C δ (ppm)
$-CH_3$	0.84 (m)	14.0
$-(CH_2)_2-$	1.22–1.26 (m)	22.5, 22.6, 29.0, 29.1, 29.3, 29.5, 29.6, 29.7, 31.5, 31.9
	1.57 (bp)	24.8
	1.96–2.03 (m)	27.1
	2.26 (t)	33.9, 34.1
$-CH{=}CH\mathbf{CH_2}CH{=}CH-$	2.72 (t)	25.6
	4.10 (dd), 4.26 (dd)	62.0
	5.22 (m)	68.9
	5.29 (m)	127.8, 128.0, 129.6, 129.8, 130.0
	–	172.5, 172.8

bp: broad peak; dd: double doublet; m: multiplet; t: triplet.

Normalized integral areas (with reference to the integral of OCH_2 protons that remains constant during the whole process) of some key protons resonances are reported in Fig. 5 *vs* ozone time treatment. Clearly, the disappearance of proton signals of **CH**=**CH** and **CH$_2$**–CH=CH–**CH$_2$** moieties follows a first order kinetics as well as the appearance of signals due to methylene protons α and β to the 1,2,4-trioxolane rings carbons. Linear regression analysis suggests that the rate of disappearance of unsaturation and the rate of formation of ozonides are almost equal (see Table 4). Such an evidence confirms that the major product of the reaction is represented by ozonides and the scission of ozonides is very minimal. Analogous results have been previously found in the analysis of sunflower oil methyl esters (Soriano et al., 2003).

3.2. Physico-chemical characterization

The physico-chemical characterization results are reported in Table 5. As far as weight increase is concerned, it appears to remain fairly constant during the ozonation process, although a slight decrease can be shown. The SO characterization confirms what has already been shown in previous studies for different vegetable fats and oils in relation to the treatment time (Diaz et al., 2001; Martinez Téllez et al., 2006; Díaz Gómez et al., 2006; Sadowska et al., 2008), namely: the decrease of double bonds (IV), the increase in peroxidic species, and the increase of viscosity. Actually, the reaction of ozone with the double bond leads to its gradual disappearance, as can be inferred by NMR analysis. The formation of peroxidic compounds, in particular 1,2,4-trioxolane, is indicated by PV. Both

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Table 3
^{1}H and ^{13}C NMR data of SO during ozone treatment as well as variation of area (^{1}H) or intensity (^{13}C) of NMR resonances with ozonation time (see text for details).

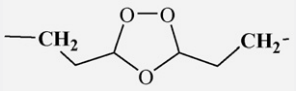
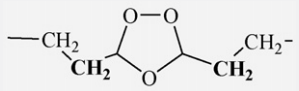
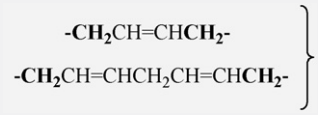
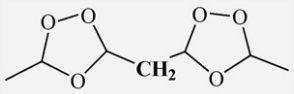
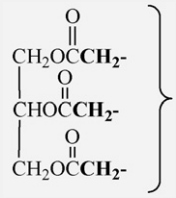
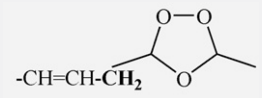
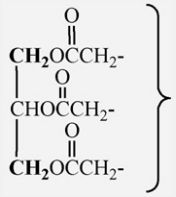
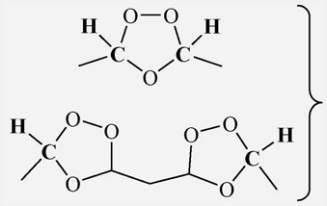
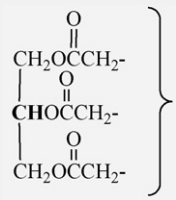
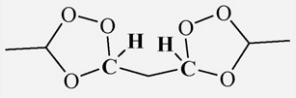
Moiety	^{1}H δ (ppm)	^{13}C δ (ppm)	Behaviour
$-CH_3$	0.82 (m)	13.8, 14.0	
$-(CH_2)_2-$	1.20–1.25 (bp)	22.3, 22.5, 28.8, 28.9, 29.1, 29.3, 29.4, 29.6, 31.2, 31.4, 31.7, 31.8, 32.3	
	1.35 (bp)	23.3, 23.4, 23.6, 23.7, 23.8	Increasing
	1.55 (bp)	24.7	
	1.63 (bp)	30.6	Increasing
	1.96–2.03 (bp)	27.1, 27.3	Decreasing
	2.04–2.12 (m)	35.6	Appearing at 60′, then increasing
	2.25 (t)	33.8, 34.0	
	2.36–2.42 (m)	41.5	Increasing (up to 75′), then decreasing
$-CH{=}CH\mathbf{CH_2}CH{=}CH-$	2.72 (t)	25.6	Decreasing
	4.10 (dd), 4.26 (dd)	62.0	
	5.08–5.11 (m)	103.4, 104.4, 104.1, 104.3	Increasing
	5.22 (m)	68.9	
	5.25–5.30 (m), 5.35–5.42 (m)	100.2, 100.4	Increasing

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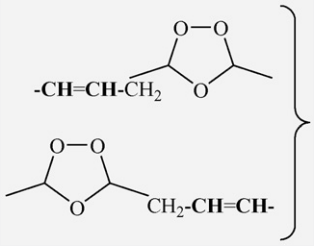
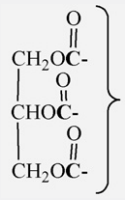
Table 3 (*Continued*)

Moiety	^{1}H δ (ppm)	^{13}C δ (ppm)	Behaviour
-CH=CH- -CH=CHCH$_2$CH=CH-	5.29 (m)	127.8, 128.0, 129.6, 129.8, 130.0	Decreasing
	5.28–5.35 (m) 5.50 (m)	120.8, 120.9, 121.3, 121.4 133.9, 134.0, 134.2	Increasing (up 75′), then decreasing
	–	172.5, 172.9	

bp: broad peak; dd: double doublet; m: multiplet; t: triplet.

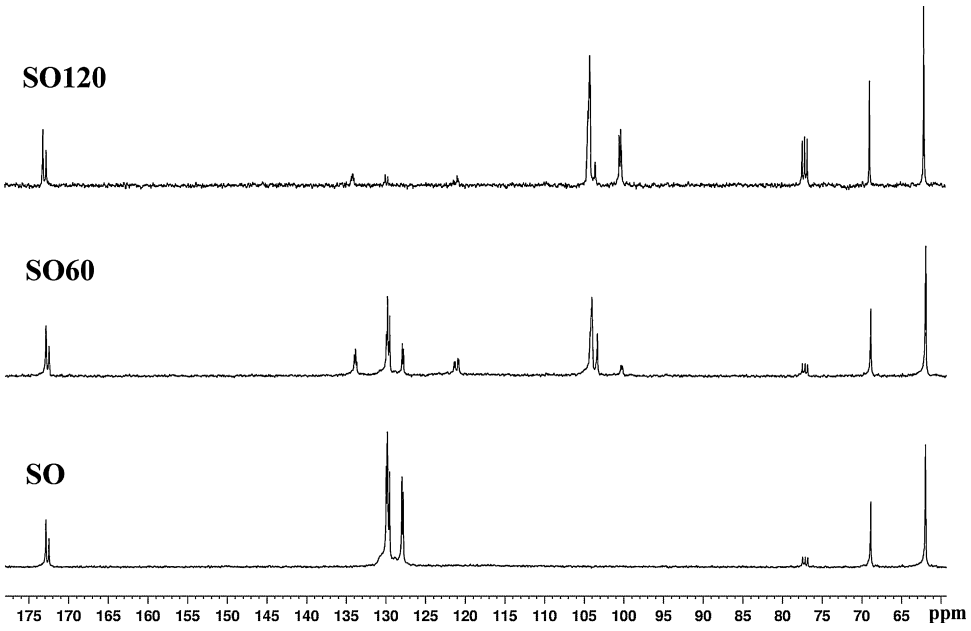


Fig. 2. ^{13}C NMR most significative spectral region of untreated SO and after 60′ (SO60) and 120′ (SO120) ozone treatment.

methods used, in fact, show an increase in the PV values in relation of ozonation time. It should be emphasized how the official method (European Pharmacopoeia, 2008b) led to an underestimation of ozone reacting with unsaturated substrates (Zanardi et al., 2008).

OE represents an estimation of the amount of ozonated compound, as evaluated by PV, to the amount of the applied ozone. As expected, the reaction yield decreases because of the double bond disappearance with time. An increase of the ozonation time leads to an exponential trend in the increase of the sample viscosity. Ozonated SO reached viscosity values up to 984 and 496 mPa s, depending upon the temperature of determination (22 and 35 °C, respectively).

Table 4
Linear regression ($y = mx + q$, r^2 parameter) of integral of selected proton chemical shifts *vs* ozonation times.

Chemical shifts (ppm)	m	q	r^2 value
1.35	0.083	0.12	0.9940
1.63	0.078	−0.17	0.9971
2.03–1.96	−0.077	10.5	0.9959
2.72[a]	−0.025	2.27	0.9920
5.11–5.08	0.032	0.020	0.9731
5.29	−0.048	6.3	0.9738

[a] Up to 90′ of ozone treatment.

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Table 5
Physico-chemical characterization of SO before and after ozonation (see text for details).

Sample	Weight increase [%]	IV [g/100 g]	PV[a] [mEq/1000 g]	PV[b] [mEq/1000 g]	OE [%]	Viscosity [mPa s]	
						22 °C	35 °C
SO	–	113.65 ± 1.50	35 ± 1	198 ± 9	–	59.9 ± 1.1	34.2 ± 0.3
SO15	103	96.05 ± 3.53	97 ± 4	949 ± 33	107	84.9 ± 0.7	48.1 ± 0.4
SO30	92	81.32 ± 2.98	165 ± 5	1631 ± 64	110	116 ± 1	64.5 ± 0.2
SO45	94	68.12 ± 3.12	229 ± 9	2281 ± 98	99	165 ± 1	92.4 ± 0.3
SO60	93	57.21 ± 2.34	262 ± 11	3170 ± 101	106	248 ± 2	129 ± 2
SO75	94	48.00 ± 2.12	297 ± 7	3520 ± 126	94	403 ± 7	194 ± 1
SO90	94	41.25 ± 2.05	313 ± 15	4134 ± 178	93	603 ± 2	297 ± 1
SO105	92	34.13 ± 1.34	325 ± 6	4312 ± 165	84	725 ± 26	380 ± 4
SO120	90	25.11 ± 1.02	332 ± 8	4542 ± 183	77	984 ± 28	496 ± 5

[a] PV as evaluated by adopting European Pharmacopoeia official monograph.
[b] PV as evaluated according to Zanardi et al. (2008).

3.3. Relationships between NMR and physico-chemical measurements

The NMR data show that a detailed correlation can be developed from conventional chemical analysis (namely, IV and PV) as well as viscosity determinations. In detail, using the NMR analysis it is possible to demonstrate the existence of a relationship between the integral values of the signals corresponding to protons which resonate at either both 5.29 and 1.97 ppm, or 5.11–5.08 ppm in the ^{1}H NMR and the IV and PV as reported in Table 4, respectively. In Fig. 6 analogous trends with respect to IV have been obtained with these selected integral values.

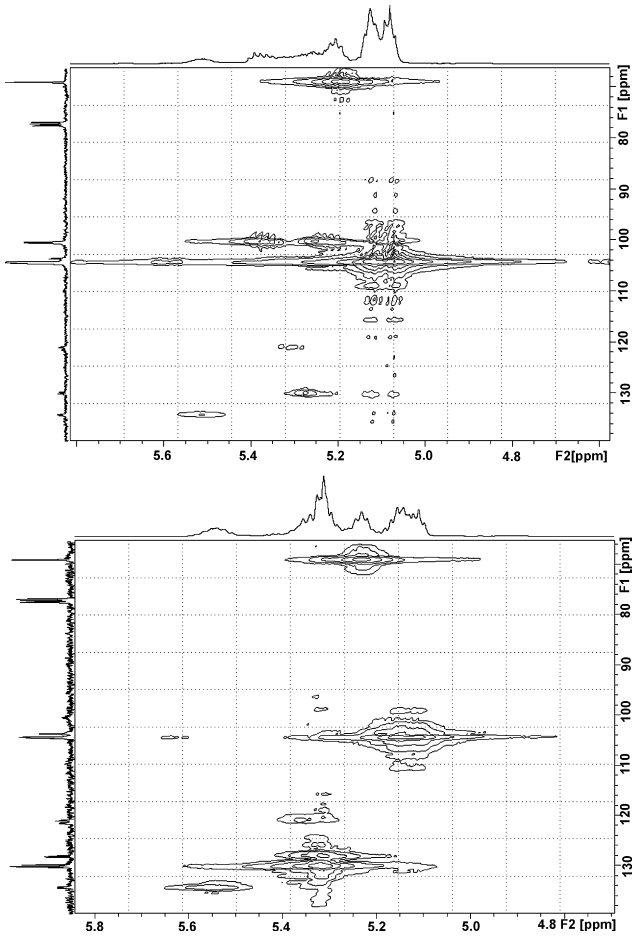

Fig. 3. HMQC spectra after 60′ (bottom) and 120′ (top) of ozonation. The most significative region is reported.

The NMR evaluation showing increasing unsaturated species disappearance coincides well with the increasing PV and viscosity values, as reported in Figs. 7 and 8, respectively. As far as PV is concerned, linear trends have been observed, even if in the modified method the OE, expressed as a percentage, decreases at higher ozonation time because of disappearance of free double bonds, as expected (Table 4 and Fig. 7). It should be highlighted that the experimental data of Fig. 7 show differences in values at exper-

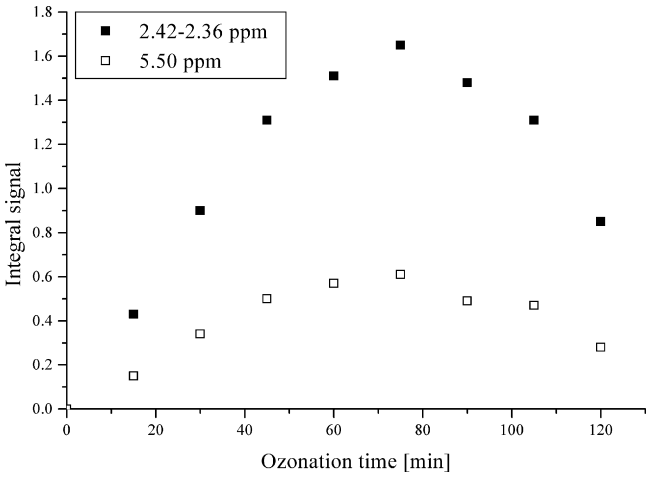

Fig. 4. Behaviour of integral areas for selected proton signals whose resonances can be accurately integrated *vs* ozonation time.

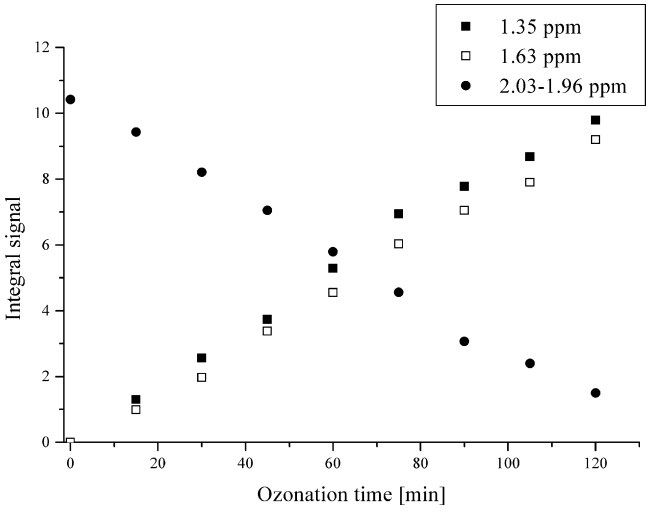

Fig. 5. Linear trends of key proton resonances whose resonances can be accurately integrated, involved in the main ozonation reaction.

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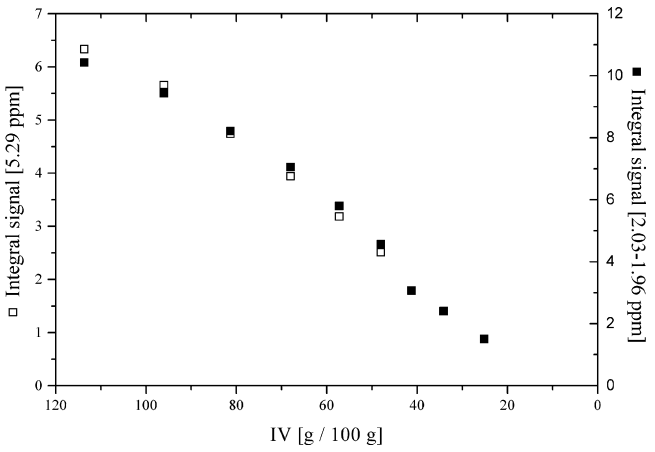

Fig. 6. Linear relationship between NMR integral signals of selected protons responsive to double bond disappearance and iodine values, IV.

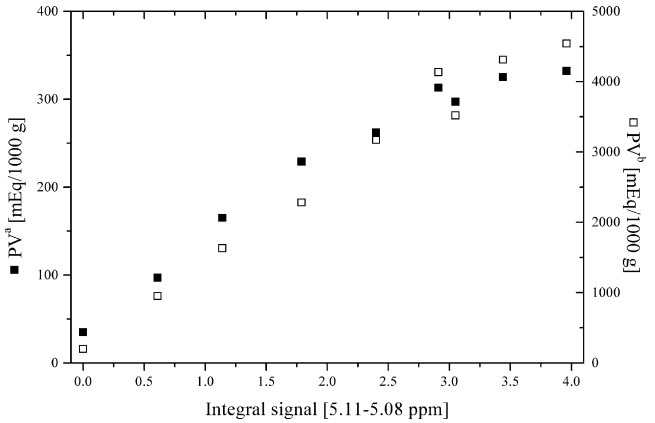

Fig. 7. Linear relationship between NMR integral signals of selected protons related to cyclic ozonated species and peroxide values, PV, as evaluated by [a]conventional method (European Pharmacopoeia, 2008b) or [b]modified one (Zanardi et al., 2008).

imental points pertinent to the full saturation of one of the two available double bonds (after 75′). Further investigations are necessary for a full correlation, but these are considered beyond the aim of the present study.

Analogous behaviour can be also observed in the viscosity evaluation: the greater the ozonation time the higher the product viscosity, with a typical exponential development when viscosity values are plotted *vs* NMR integral areas related to the disappearance of double bond signals (Fig. 8). For the latter, to better describe their inversely proportional trends with respect to the ozonation process a reverse scale has been adopted. According to these observations, Eq. (2) can be written as:

$$\eta = \eta_{sat} \cdot e^{k \cdot IS} \quad (2)$$

where η indicates the experimental values of viscosity at the different ozonation times; η_{sat} is the estimated viscosity at theoretical full double bond saturation; IS represents the NMR integral values of the corresponding signals and k is an experimental constant. Specifically, for the experimental data as obtained at 22 °C (Table 4) very similar values for η_{sat} have been obtained, namely 1563 ± 27.7 and 1558 ± 210 mPa s for the integral signals related to 2.03–1.96 and 5.29 ppm, respectively. Correspondingly, k values of $-0.3113 \pm 6.2E{-}3$ and $-0.5528 \pm 4.3E{-}2$ ppm^{-1} have been estimated. Analogous fitting analysis and akin results could be achieved at different temperatures of viscosity determination (data not shown). Such an equation is, therefore, a useful tool in providing a rapid quality control assessment during the entire ozonation process.

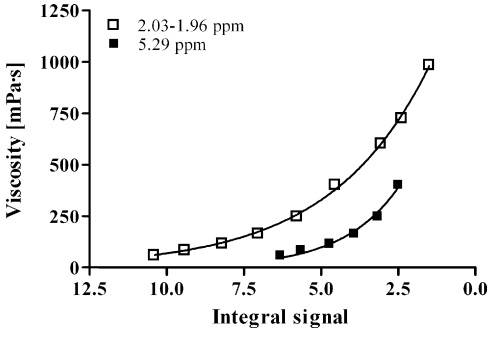

Fig. 8. Typical exponential plots for viscosity values and NMR integral signals of selected protons responsive to double bond disappearance. See text for details.

In conclusion, the NMR results showed that SO ozonation in the experimental conditions described was a smooth process giving rise almost exclusively to ozonated species: mono-ozonates from oleic acid component, and di-ozonates from linoleic acid component of triglycerides. Secondary oxidation products like aldehydes or fatty acids or peroxides were practically absent. These facts are consistent with:

(i) Almost complete ozonation of double bonds.
(ii) Effectiveness of NMR spectroscopy in following and analyzing the species developing during the ozone reaction with SO.
(iii) Reliability and reproducibility of the industrial ozonation process, leading to very good quality ozonated SO for pharmaceutical purposes.

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References

Criegee, R., 1975. Mechanism of ozonolysis. Angew. Chem. Int. Ed. 14, 745–752.
Diaz, M., Lezcano, I., Molerio, J., Hernandez, F., 2001. Spectroscopic characterization of ozonides with biological activity. Ozone-Sci. Eng. 23, 35–40.
Díaz Gómez, M.F., Téllez, G.M., Cruz, M.A., Mancheño, R.G., 2006. Chemical analysis of ozonized theobroma fat. J. Am. Oil. Chem. Soc. 83, 943–946.
European Pharmacopoeia, 2008a. Council of Europe. Strasbourg, France, pp. 2874–2875.
European Pharmacopoeia, 2008b. Council of Europe. Strasbourg, France, pp. 138–139.
Giordano, A., Vella, F.M., Romano, I., Gambacorta, A., 2007. Structural elucidation of a novel phosphoglycolipid isolated from six species of *Halomonas*. J. Lipid Res. 48, 1825–1831.
Guillén, M.D., Goicoechea, E., 2007. Detection of primary and secondary oxidation products by Fourier transform infrared spectroscopy (FTIR) and ^{1}H nuclear magnetic resonance (NMR) in sunflower oil during storage. J. Agric. Food Chem. 55, 10729–10736.
Horváth, M., Bilitzky, L., Hüttner, J.M.L., 1985. Fields of utilization of ozone. In: Clark, R.J.H. (Ed.), Ozone. Elsevier Science Publishing Co., New York, USA, pp. 257–316.
Li, C.Y., Tsai, S.I., Damu, A.G., Wu, T.S., 2009. A rapid and simple determination of protoberberine alkaloids in Rhizoma Coptidis by ^{1}H NMR and its application for quality control of commercial prescriptions. J. Pharm. Biomed. Anal. 49, 1272–1276.
Martinez Téllez, G., Lozano, L.O., Díaz Gómez, M.F., 2006. Measurement of peroxidic species in ozonized sunflower oil. Ozone-Sci. Eng. 28, 181–185.
Pryor, W.A., Das, B., Church, D.F., 1991. The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. Chem. Res. Toxicol. 4, 341–348.
Retief, L., McKenzie, J.M., Koch, K.R., 2009. A novel approach to the rapid assignment of ^{13}C NMR spectra of major components of vegetable oils such as avocado, mango kernel and macadamia nut oils. Magn. Reson. Chem. 47, 771–781.
Sadowska, J., Johansson, B., Johannessen, E., Friman, R., Broniarz-Press, L., Rosenholm, J.B., 2008. Characterization of ozonated vegetable oils by spectroscopic and chromatographic methods. Chem. Phys. Lipids 151, 85–91.

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Soriano Jr., N.U., Migo, V.P., Matsumura, M., 2003. Ozonation of sunflower oil: spectroscopic monitoring of the degree of unsaturation. J. Am. Oil Chem. Soc. 80, 997–1001.
Thornberry, T., Abbatt, J.P.D., 2004. Heterogeneous reaction of ozone with liquid unsaturated fatty acids: detailed kinetics and gas-phase product studies. Phys. Chem. Chem. Phys. 6, 84–93.
Travagli, V., Zanardi, I., Boschi, L., Gabbrielli, A., Mastronuzzi, V.A., Cappelli, R., Forconi, S., 2008. Comparison of blood viscosity using a torsional oscillation viscometer and a rheometer. Clin. Hemorheol. Microcirc. 38, 65–74.
Travagli, V., Zanardi, I., Bocci, V., 2009. Topical applications of ozone and ozonated oils as anti-infective agents: an insight into the patent claims. Recent Pat. Anti-infect. Drug Discov. 4, 130–142.
Zanardi, I., Travagli, V., Gabbrielli, A., Chiasserini, L., Bocci, V., 2008. Physico-chemical characterisation of sesame oil derivatives. Lipids 43, 877–886.
Zhou, Z., Ribeiro, A.A., Raetz, C.R.H., 2000. High-resolution NMR spectroscopy of lipid A molecules containing 4-amino-4-deoxy-L-arabinose and phosphoethanolamine substituents. J. Biol. Chem. 275, 13542–13551.

Please cite this article in press as: Sega, A., et al., Properties of sesame oil by detailed ^{1}H and ^{13}C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements. Chem. Phys. Lipids (2009), doi:10.1016/j.chemphyslip.2009.10.010