Physico-Chemical Characterization of Sesame Oil Derivatives

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ORIGINAL ARTICLE

~~Physico-chemical characterisation~~Physico-Chemical Characterization of ~~sesame oil derivatives~~Sesame Oil Derivatives

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Abstract Ozone treatment of ~~commercially available~~commercially available vegetable oils gives rise to the formation of chemical species ~~which~~that are responsible for the ~~therapeutical~~therapeutic properties of ozonated oil derivatives in dermatological diseases. ~~During~~In the last years, these products have been successfully used as a topical disinfectant in a number of serious skin affections. The medical application of empirically prepared ozonated oil has yielded striking improvements with unexpected and rapid ~~healing to compel~~healing, compelling us to begin a long-range study aiming first to ~~firstly~~ define the main characteristics of the most common ozonated vegetable ~~oils that~~oils, about which there is usually ~~do not obtain~~no medical consensus ~~due to~~because of the lack of standardization of their technological parameters. Sesame oil was selected because of its great amount of polyunsaturated acyl groups, as well as ~~of~~ natural antioxidants. Moreover, we have determined the kinetics and optimal conditions of ozonation ~~(eg~~(e.g., ozone concentrations, time of exposure, temperature) for obtaining an ozonated oil characterized by well-established technological and ~~physicochemical~~physico-chemical properties, namely an accurate peroxide value determination. On the basis of the results, we have gained an understanding of the modifications of the vegetable oils during the ozonation process.

Keywords Oxidized lipids · PUFAs · Analytical techniques

Abbreviations

~~(4-HNE)~~4-HNE	~~4-hydroxynonenal~~4-Hydroxynonenal
~~(AV)~~AV	~~acid~~Acid value
~~(IV)~~IV	~~iodine~~Iodine value
~~(PV)~~PV	~~peroxide~~Peroxide value
~~(OE)~~OE	~~ozonation~~Ozonation efficiency

~~(PUFAs)~~PUFAs

~~(SO)~~SO

~~(TAG)~~TAG

~~polyunsaturated~~Polyunsaturated fatty acids

~~sesame~~Sesame oil

~~triacylglicerides~~Triacylglicerides

Introduction

From many years the medical use of ozone has represented a challenging topic [1–5]. The parenteral ~~autohemotherapy – namely~~autohemotherapy—namely the method of exposing *ex vivo* human blood to oxygen–ozone for a few minutes in an ozone-resistant glass bottle and successive, prompt reinfusion in the donor ~~patient – is~~patient—is still the object of controversial findings [6]. It has been reported that ozonetherapy is very useful in many ~~pathologies like~~pathologies, such as chronic osteomyelitis, pleural empyema, abscesses with intractable fistulae, infected wounds, bed sores, chronic ulcers and initial gangrene, necrotizing fasciitis, diabetic foot, skin, mouth, vaginal and rectal bacterial as well as viral infections and burns [7], especially when in combination with topical therapy by ozonated oils [8, 9]. Rapid healing is attributable to the disinfectant properties of ozone and to an enhanced cell proliferation allowed by an improved oxygenation and metabolism [10]. Thus, the antimicrobial properties of ozone derivatives like ozonated oils represent an interesting pharmaceutical approach to the management of a variety of dermatological pathologies [10, 11]. Unsaturated vegetable oils react with ozone at the level of double bonds to form ozonoid compounds that have sustained germicidal actions, as well as retaining the properties of stimulating tissue regeneration and repair.

The chemical reactions of ozone when bubbled into an oil are very complex. The analyses of these reactions provide information on the functional group changes during ozonation as well as the identification of the products without prior separation techniques, according to the well-known Criegee mechanism regarding the formation of ozonides from alkenes and ozone [12]. The peroxidic species are one of the most important products formed. This group includes hydroperoxides, hydrogen peroxides, polymeric ~~peroxides~~peroxides, and other organic peroxides [13–15]. The yield of ozonated derivatives from unsaturated oils depends on reaction conditions ~~(eg~~(e.g., temperature, time, ozone generator, reactor type, stirring conditions, applied ozone dose). Therefore, ~~the knowledge~~an understanding of the ~~physicochemical~~physico-chemical properties of ozonated vegetable oils has ~~a~~ great importance for their characterization and identification. For determining the quality of ozonated products, analytical methods such as peroxide, acidity, and iodine values are usually carried out [10, 13, 15~~]~~].

Referring to ozonation of vegetable raw materials, theobroma ~~fat~~fats [16] as well as olive, soybean and sunflower oils have been the most investigated [17–19]. The aim of the present study was to extend knowledge of pharmaceutical interest to other vegetable oil products, with particular emphasis on sesame oil (SO). It is expressed from the seeds of *Sesamum indicum*. SO is used in the pharmaceutical, cosmetic, and food industries. The major use of SO in pharmaceutical formulations is as a solvent in the preparation of sustained-release intramuscular or subcutaneous injections [20, 21] as well as an auxiliary substance in the preparation of oral capsules, disperse ~~systems~~systems, and topical ointments [22, 23]. SO has been selected for our investigation because it is a convenient starting material, with a high concentration of olefinic double bonds. SO is composed principally of triacylglicerides (TAG). The predominant unsaturated fatty acids in SO are oleic acid (45.4%) and linoleic acid (40.4%). However, these acyl groups are not distributed randomly across the glycerol positions. The saturated acyl groups are almost exclusively in the 1 and 3 ~~positions~~positions, and unsaturated acyl groups are predominantly in the 2 position [24]. Furthermore, SO presents significant proportions of natural antioxidants, which protect it against natural oxidation [25, 26] and could play a significant role during the ozonation process. Our goal is to investigate the doses of ozone, which, at appro-

priate experimental conditions, can react with oils leading to biologically active derivatives without any deleterious effect in terms of toxicity and stability. Moreover, attention should be paid to a correct quantification of the peroxide ~~value should be expended,~~value, because of the great ~~amount~~number and stability properties of the various peroxidic species generated during ozone treatment [14] as well as the underestimated values obtained using official methodology.

We consider our results useful to determine technological aspects that are necessary for the optimization of ozonated oil quality.

Experimental ~~procedure~~Procedure

Materials

Chemicals were purchased from Sigma-Aldrich and used without further purification. In ~~particular~~particular, the SO was obtained from the seeds of ~~*Sesamum*~~*S. indicum* (batch number S3547). ~~4-hydroxynonenal~~4-Hydroxynonenal (4-HNE) was obtained from Cayman Chemical Co. and stored at ~~−80 °C.~~−80°C.

Ozonation ~~method~~Method

Ozone was generated by the O_3 generator (Model Ozonosan PM ~~100 K,~~100 K, Hansler GmbH, Iffezheim, Germany) from medical-grade oxygen (O_2) as feed gas using electrical corona arc discharge. The gas flow rate and O_3 concentration have been controlled in real time by both photometric and iodometric determinations, as recommended by the ~~Standardisation~~Standardization Committee of the International O_3 Association. The O_3 ~~flow rate~~flow rate was kept constant at 1.5 ~~L/min~~l/min in all ~~experiments~~experiments, and ozone concentration was ~~55 mg/L. Ozone/Oxygen~~55 mg/l. The ozone/oxygen mixture was bubbled in Drechsel bottles containing ~~40 mL~~40 ml of SO for different times ~~comprised~~comprising between 15 and ~~120 min~~120 min (SO15, SO30, SO60, SO90 and SO120), and corresponding to about ~~1240, 2480, 4950, 7450~~1,240, 2,480, 4,950, 7,450 and ~~9900 mg~~9,900 mg of O_3 dose, respectively. Gas inlet was performed by using a Drechsel bottle with a sintered filter at the bottom (porosity ~~145–174 lm),~~145–174 lm), unless otherwise stated. Furthermore, we have carried out blank tests under the same conditions bubbling only oxygen for the same time (see above).

~~FT-IR spectroscopy~~FT–IR Spectroscopy

Ozonated SO samples were used for ~~FT-IR~~FT–IR spectroscopy measurement (Perkin–Elmer Spectrum BX). About ~~2 lL~~2 ll of sample were deposited between two disks of KBr, avoiding air bubble formation [27], then the transmittance % was measured in the range ~~4000–800 cm~~4,000–800 cm^{-1}. Spectra were obtained using a ~~16-scan~~16-scan summation at a minimal resolution of ~~4 cm~~4 cm^{-1}.

NMR ~~spectroscopy~~Spectroscopy

^1H and ^{13}C NMR spectroscopy was performed on a Bruker Avance ~~400 MHz Spectrometer. 100 lL~~400-MHz Spectrometer; 100 ll of each sample ~~were~~was placed into ~~5 mm tube~~5-mm tubes and dissolved in $CDCl_3$ ~~(750 lL).~~(750 ll). To obtain information on the production of toxic aldehydes like 4-HNE as by-products of ozonation, a neat SO sample was cured with ~~5 ll~~5 ll of ~~4-HNE~~4-HNE, and the ^1H NMR was recorded.

Iodine ~~value~~Value

The iodine value (IV) is a measure of the total number of double bonds present in the sample. It represents the quantity of iodine (in grams) that will react with the double bonds in ~~100 grams~~100 g of sample. IV was determined according to the European Pharmacopoeia [28]. The IV was calculated by means of the following equation:

$$IV = \frac{1.269 \cdot (n_1 - n_2)}{m} \quad (1)$$

where n_1 is the volume in ~~mL~~ml of thiosulphate solution used for carry out a blank test, n_2 is the volume in ~~mL~~ml of thiosulphate solution used for the ~~titration~~titration, and m the quantity, in grams, of substance.

Acid ~~value~~Value

The acid value (AV) is the number that expresses, in mg, the quantity of potassium hydroxide required to ~~neutralise~~neutralize the free acids ~~presents~~present in ~~1 g~~1 g of the substance [29]. The AV was ~~calculate~~calculated by means of the following equation:

$$AV = \frac{5.610 \cdot n}{m} \quad (2)$$

where n is the volume in ~~mL~~ml of titrant and m the quantity, in grams, of substance.

Peroxide ~~value~~Value and ~~method evaluation~~Method Evaluation

The peroxide value (PV) represents the quantity of peroxide expressing in milliequivalents of active oxygen contained in ~~1000 g~~1,000 g of the sample.

PV was determined introducing changes, unless otherwise stated, into the method described in the official monograph [30], as recently evidenced by some authors in the case of materials characterized by a high peroxide content and slow iodide reactivity with dialkylperoxides [14, 31]. In particular, we are able to obtain reproducible and/or realistic results only by modifying both the temperature and the reaction time with respect to the monograph conditions. Briefly, ~~2 g~~2 g of SO were weighed in a ~~250 mL~~250-ml conical ~~flask~~flask, and ~~30 mL~~30 ml of chloroform/glacial acetic acid (2:3) were added. Then, ~~3.0 mL~~3.0 ml of saturated potassium iodide solution ~~were~~was added. The flask was stirred at reflux temperature ~~(60°C)~~(60° C) for various times ~~(0–120 minutes).~~(0–120 min). After this time, the solution was ~~cooled~~cooled, and ~~25 mL~~25 ml of water ~~were~~was added. Solutions of sodium thiosulphate at the appropriate concentration ~~(0.0001–0.1 M)~~(0.0001–0.1 M) were used for the titration. The PV was calculated by means of the following equation:

$$PV = \frac{1000 \cdot (V_1 - V_0) \cdot c}{m} \quad (3)$$

where V_1 is the volume in ~~mL~~ml of thiosulphate solution used for the titration, V_0 is the volume in ~~mL~~ml of thiosulphate solution used for carry out a blank, c the thiosulphate ~~concentration~~concentration, and m the quantity of substance.

All samples carried out by peroxide value titration were treated in order to evaluate the reaction process. Briefly, the organic layer of titrated mixture was dried over sodium sulfate and evaporated under reduced pressure to give a yellow oil, which ~~has been~~was analyzed by both ~~FT-IR~~FT–IR and ^{1}H-NMR spectroscopies (see above).

Moreover, the ozonation efficiency (OE%) was calculated. It represents the % ratio of the amount of peroxidation due to the ozonation process, as estimated by PV value, to the amount of total ozone applied to the system.

Viscosity ~~measurements~~Measurements

Viscosity measurements were carried out with an innovative torsional-oscillation viscometer [32] (Viscomate VM-10AL, CBC Europe). Briefly, this ~~instruments measure~~instrument measures viscosity by sensing a change in oscillation amplitude of a liquid-immersed detector, based on constant input voltage. The detector oscillation amplitude with no resistance is ~~1 lm.~~1 lm. Angular acceleration of the detector is measured and reported as dynamic viscosity with a declared range of ~~0.400—1000 mPa.s~~0.400–1,000 mPa s and a precision equal ~~to ± 5%.~~to ±5%. All the determinations were conducted in polystirene Technicon® sample cups (Kartell, nominal capacity ~~2 mL).~~2 ml). Temperature control was accurately monitored during the experiments ~~(22.0 ± 0.1~~(22.0 ± 0.1 or ~~35.0 ± 0.1 °C).~~35.0 ± 0.1 C). Viscosity values were recorded until equilibrium (data collection every ~~5 s)~~5 s) by PC connection through a RS-232 port.

In the present study all the physico-chemical ~~characterisations~~characterizations were repeated at least three times.

Results

~~FT-IR spectroscopy~~FT–IR Spectroscopy

For the IR spectroscopy of SO we have focused our attention on the ~~wavenumbers~~wave numbers characterizing the double bond, ~~C = C~~C = C stretching ~~(1654 cm~~(1,654 cm^{-1}) ~~and = C-H~~and = C–H stretching ~~(3009 cm~~(3,009 cm^{-1}) [33], as well as the ozonide CO stretching (the ~~1105 cm~~1,105 cm^{-1}) by unsaturated fatty acid moieties [34, 35]. As expected, the intensity of the bands corresponding to the double bond decreases and the band identifying the formation of the ozonide increases with respect to time of ozone treatment (Fig. 1). Apparently, when we have the maximum ozone dose, all double bond signals disappeared. As can be seen, the peaks ~~above~~ reported above reveal the chemical changes occurring ~~to~~in the fatty acid chain during the reaction.

NMR ~~spectroscopy~~Spectroscopy

The ^{1}H NMR and ^{13}C NMR assignment to principal peaks of SO ~~were~~are listed in Table 1. The proton and carbon resonances were assigned according with literature data, as obtained for similar vegetable oils [25, 34]. The ^{1}H NMR spectrum of SO is shown in Fig. 2 ~~(top).~~, top. NMR analyses confirmed the structural changes undergone by oil

Table 1 ^{1}H and ^{13}C NMR assignments of neat sesame oil (SO)

Chemical shift (ppm)		Functional group
^{1}H	^{13}C	
0.83	14.28	~~-CH-~~**CH_3**
1.27	~~29.15-31.89~~29.15–31.89	~~(-~~**$(CH_2)_n$**~~-~~–
1.60	24.84	~~-CH2-~~**CH_2**~~-CH2-OCO-~~CH_2–OCO–
2.00	27.18	~~-CH-~~**CH_2**~~-CH = CH-~~–CH = CH–**CH_2**~~-~~–
2.30	34.08	~~-CH-~~**CH_2**~~-OCO-~~–OCO–
2.71	25.62	~~-CH = CH-~~–CH = CH–**CH_2**~~-CH = CH-~~–CH = CH–
~~4.05-4.35~~4.05–4.35	173.07	~~-CH-~~**CH_2**~~-OCOR-~~–OCOR
~~5.20-5.26~~5.20–5.26	172.73	> **CH**~~-OCOR-~~–OCOR
~~5.20-5.38~~5.20–5.38	~~128.06-130.16~~128.06–130.16	~~-CH-~~–CH_2~~-~~ ~~CH-CH=-~~ = ~~CH-~~**CH**–CH_2~~-~~–
~~-~~–	172.98	~~-OC-~~**OC**~~O-~~O–

during the ozonation (Fig. 2, bottom). The new signals found in ozonated oil are summarized in Table 2. In ^{1}H NMR spectra of ozonated ~~SO~~SO, new signal at ~~5.15 ppm~~5.15 ppm appears, attributed to the ring proton of 1,2,4-trioxolane. Such ~~an~~ evidence was confirmed by the appearance of signal in ^{13}C NMR spectra at ~~104.33 ppm~~104.33 ppm assigned by Wu et al. [36] as the ring carbon in the same structure. ~~Moreover~~Moreover, the decrease of multiplet at ~~5.20–5.38 ppm~~5.20–5.38 ppm and of signals at ~~128.06–130.16 ppm~~128.06–130.16 ppm was observed in the ^{1}H and ^{13}C NMR ~~spectra~~spectra, respectively, due to olefinic moiety. Furthermore, a new signal at ~~5.56 ppm,~~5.56 ppm, assigned as protons directly bonded to sp^{2}-hybridized carbons, appeared at the early stage of ozonation (until ~~60 min)~~60 min) to gradually decrease, in agreement with previous results obtained for transesterified sunflower oil [34].

The protons of the methylene group a to the sp^{2}–hybridized carbons resonated at 2.00 and ~~2.71 ppm~~2.71 ppm before the ~~ozonation,~~ozonation and shifted to 1.36 and ~~1.65 ppm~~1.65 ppm after formation of 1,2,4-trioxolane [37]. ^{1}H NMR spectra of ozonated SO showed peaks at 9.69 and ~~2.42 ppm~~2.42 ppm attributable to the aldehydic proton and the methylene ~~protons~~proton a to the carbonil carbon, respectively. In ^{13}C NMR, the carbonil ~~carbon~~carbon, and the carbon a to it, resonated at 202.58 and ~~44.02 ppm,~~44.02 ppm, respectively.

Iodine, Acid and Peroxide Values

In Table 3 the IV, AV, PV as well as the ozonation efficiency are reported. As far as IV is concerned, it decreases as the insufflated ozone dose ~~increases~~increases, leaving from the value of 113.65 (according to certificate of analysis limits, 103–116) to 13.39 with the greater ozone dose used ~~(9900 mg).~~(9,900 mg). Such a value is indicative that about all of the unsaturated group in SO reacted with ozone, according to the double bond disappearance.

An increase in both acidity and peroxidation values was observed. In detail, about 18- and 34-fold grow-up with respect to the initial value and ozonation time ~~were~~was obtained, respectively (see Table 3). In the first ozonation phase (SO30, ~~2480 mg~~2,480 mg of ozone) the increase ~~is~~was fast, followed by a decrease in rate, until the plateau of about ~~3800~~3,800 was obtained in terms of peroxide value (SO120, ~~9900 mg~~9,900 mg of ozone). These results were expected because, in preliminary experiments, we noted that only a partial reaction of ozone in the oil compartment as evidenced by an increased amount of ozone recovered in a suitable iodide trap was posed after the vessel. Such a phenomenon is justified if we consider that a substantial portion of the unsaturation became slow reacting with time, because of probabilistic and steric conditions [38]. This ~~behaviour~~behavior might be due to formation of polymeric ~~peroxides~~peroxides, which are responsible for viscous mass achieved after massive ozonation.

In Table 3, ozonation efficiency was also reported. It represents an estimation of the amount of ozonated compound, evaluated by PV, to the amount of the applied ozone. As expected, the reaction yield decreases because of the double bond disappearance with time.

Table 2 ^{1}H and ^{13}C NMR new signals of ozonated sesame oil

Chemical shift (ppm)		Functional group
^{1}H	^{13}C	
5.15	104.33	1,2,4-trioxolane
1.36	–	~~–CH–~~ **CH_2** ~~–CH = CH–~~ CH = CH ~~–CH–~~ **CH_2** ~~–~~
1.65	–	~~–CH = CH–~~ CH = CH– **CH_2** ~~–CH = CH–~~ CH = CH–
2.42	44.02	a-Methylene group
9.69	202.58	Aldehyde

Table 3 Iodine ~~Value~~value (IV), ~~Acid Value~~acid value (AV) and ~~Peroxide Value~~peroxide value (PV) of the samples after exposure to progressively increasing ozone amounts

Sample	*Ozone dose* ~~[mg]~~(mg)	*IV* ~~[g / 100 g]~~(g/100 g)	*AV* ~~[mg KOH / g]~~(mg KOH/g)	*PV* ~~[mEq / 1000 g]~~(mEq/1,000 g)	*OE* ~~[%]~~(%)
SO	0	113.65 ~~(± 1.50)~~(±1.50)	0.70 ~~(± 0.01)~~(±0.01)	104 ~~(± 3)~~(±3)	~~–~~–
SO15	~~1240~~1,240	92.20 ~~(± 1.60)~~(±1.60)	2.30 ~~(± 0.08)~~(±0.08)	~~1480 (± 70)~~1,480 (±70)	96
SO30	~~2480~~2,480	79.65 ~~(± 4.80)~~(±4.80)	3.19 ~~(± 0.08)~~(±0.08)	~~2503 (± 101)~~2,503 (±101)	84
SO60	~~4950~~4,950	52.65 ~~(± 1.10)~~(±1.10)	7.40 ~~(± 0.18)~~(±0.18)	~~3100 (± 93)~~3,100 (±93)	52
SO90	~~7425~~7,425	37.10 ~~(± 1.00)~~(±1.00)	9.80 ~~(± 0.30)~~(±0.30)	~~3440 (± 89)~~3,440 (±89)	38
SO120	~~9900~~9,900	13.90 ~~(± 0.50)~~(±0.50)	12.6 ~~(± 0.35)~~(±0.35)	~~3800 (± 179)~~3,800 (±179)	31

Method ~~evaluation~~Evaluation of the Peroxide Value

The PV determination according to the European Pharmacopoeia [30] leads to an underestimation of O_3 reacting with unsaturated substrates. In fact, these values are very low with regard to the O_3 dose insufflated ~~(PV < 350,~~(PV < 350, see ~~Figure~~ Fig. 3). The difference between values among the various treatment times is minimal, even if statistically significant ~~(p < 0.001),~~($P < 0.001$), in all cases except between SO90 and SO120. ~~FT-IR~~FT–IR spectra of titrated samples confirm this assumption: ozonide CO stretching ~~(1105 cm~~(1,105 cm^{-1}) was unchanged between the control sample and the sample titrated according to Pharmacopoeia ~~(Figure~~ (Fig. 4). Such a result was also validated by the signal at ~~5.15 ppm~~5.15 ppm in NMR spectra (data not shown).

The ~~behaviour~~behavior is different if the sample was stirred at reflux temperature: PV considerably increases already after ~~10 min~~10 min (about ~~2800~~2,800 for SO120), corresponding to a decrease in the CO stretching band. The full titration was carried out for ~~180 min,~~180 min at reflux. The plateau, defined as the absence of ~~statistical~~ statistically significant differences between two successive points, was reached after ~~120 min~~120 min for SO15, SO30, and SO60, whereas the samples SO90 and SO120 required minor reflux times ~~(30 min).~~(30 min).

The data ~~are~~were confirmed by NMR spectroscopy analysis carried out on oil recovered from the titration, where the ~~5.15 ppm~~5.15 ppm signal disappears.

It should be noted that the heat treatment does not change the ~~FT-IR~~FT–IR transmittance band of the SO control sample ~~(Figure~~ (Fig. 4, left side).

As expected, the OE% is almost quantitative ~~(> 96%)~~(> 96%) and anyhow high ~~(> 80%)~~(> 80%) for SO15 and SO30, respectively. After this time, it is possible to observe a reduction in the ozone yield (until 31%, SO120) due to a lack in the number of available double bonds for the reaction (Table 3).

Viscosity ~~measurement~~Measurement

An increase of the ozonation time leads to an exponential trend in the increase of the sample viscosity, as shown in Fig. 5. Ozonated SO reached viscosity ~~value~~values up to 900 and ~~400 mPa·s,~~400 mPa s, depending to the temperature of determination (22 ~~°C~~ and 35 °C, respectively).

It is noteworthy that when ozone inlet was performed by using a Drechsel bottle with Drechsel bottle heads in the absence or in the presence of a sintered filter disk, analogous results in terms of IV, PV NMR and ~~FT-IR~~FT–IR characterization as well as viscosity determination were always obtained. Moreover, when oxygen inlet instead of ozone was performed as control, no differences were observed with respect to the native SO characteristics.

Eventually, the stability of the ozonated oil was monitored over a period of at least ~~1 year.~~1 year. No appreciable changes in the physico-chemical profile of the derivatives was ~~observed,~~observed if they were stored at room ~~temperature,~~temperature in the dark.

Discussion

SO represents a valuable matrix to obtain derivatives useful in treating dermatological affections. SO was selected because this vegetable product is often used in pharmaceutical formulations, but it was never characterized in terms of its reactivity with ozonation processes. Our proposed accurate methods to check the oxidative processes and status are important factors in terms of sample stability.

Specific bibliographic information on the spectroscopic characterization ~~(FT-IR~~(FT–IR and NMR) of SO derivatives generated by ozonation is absent. Ozone concentration and time of reaction are important for the ozonation reaction. The appreciable change in the ~~FT-IR~~FT–IR spectra between ~~3300~~3,300 and ~~3600 cm~~3,600 cm^{-1} broad ~~peak~~peak, indicating the presence of hydroxyl groups in the ozonated oil different ~~than~~from SO [35], was not evidenced in our study.

The extent of ozonation had very little effect on the ~~aldehyde-to-ozonide~~aldehyde-to-ozonide ratio obtained from NMR spectra. These results indicate that, as expected, also for SO the NMR spectroscopy can provide valuable information about the amount of reaction compounds during ozone treatment. ^{1}H-NMR spectra of neat oil samples and ozone-treated oil showed peaks due to methyl protons from fatty acids. It was also evidenced from NMR spectra that the double bond peak decreased in intensity after ozonation. Moreover, the appearance of new peaks at 2.42 and ~~9.69 ppm~~9.69 ppm for the ozonated oils ~~are~~is comparable with analogous derivatives ~~obtains~~obtained for similar substances (2.50 and ~~9.75 ppm).~~9.75 ppm). These results can be explained by the decomposition of all the peroxidic compounds present in the different ~~equilibriums,~~equilibriums into the same reaction system. One example of these is the formation of carboxylic acid from peroxidic compounds. Besides, SO has a high proportion of unsaturated fatty ~~acid~~acid, leading to a very complex ozonated system where the peroxidic compound decomposition to acid is very high.

As far as formation of several secondary oxidation products is concerned, aldehydes are of peculiar concern in terms of their cytotoxic and genotoxic potentiality [39]. In particular, among the reactive species produced upon lipid peroxidation, 4-HNE is one of the most abundant aldehydes when ozone reacts with olefins in the presence of water [40]. In the overall ~~spectra~~spectra, it was observed that the specific region of this compound ~~(9.57–9.59 ppm)~~(9.57–9.59 ppm) was free of signals, leading us to conclude that 4-HNE is not a secondary product of SO ozonation.

During ozonation reaction, ozone reacted with the vegetable oils ~~which~~that have high levels of polyunsaturated fatty acids (PUFAs). This aspect was evidenced by the IV decrease in relation to applied ozone dosage, representing a measure of the residual double bonds. Almost all unsaturated groups reacted with ozone, even if the condensation of peroxides led to a thickening of the system that could limit the whole double bond accessibility [19, 24].

The reaction of ozone with PUFAs present in ~~SO~~SO, as well as in other unsaturated substrates, produces different types of peroxidic compounds as described by the Criegee mechanism, and the peroxide value represents the method generally employed to determine total peroxidic compounds formed. However, the official monographs report standard methods based on iodometric characterization with a reaction time of ~~one minute~~1 min to analyze the samples. It ~~is to observe~~should be observed that these assays ~~well~~ apply well only to samples at low content of peroxidic compounds. In fact, ozonated SO as well as other unsaturated oils contains a great amount and diversity of peroxides. For these reason, different conditions could be necessary to determine its correct peroxide content. In particular, the prescribed reaction time of ~~one minute~~1 min is insufficient for determining all kinds of long-chain peroxides [14, 31].

Acids are directly formed both during ozonation and because of peroxide decomposition. ~~However~~However, at these conditions, a high acidity value does not directly indicate that the oil quality has diminished and is at risk of becoming rancid.

Viscosity represents an evaluation of the peroxides species that are generated during the SO ozonation process, giving information about: ~~i)~~(1) the increase of the van der Waals interactions due to the disappearance of the double bonds, indicating that the double bonds in the oil molecules reacted with ozone to form a more bulky molecule; ~~ii)~~(2) the modification of the unsaturated acyl chains ozonation kinetic, affecting the mobility and the reactivity of the species involved in the reaction. This aspect is of particular importance in terms of product characterization and evaluation of the ozonation reaction. Furthermore, our proposed method represents a useful online instrumentation that doesn't require periodic recalibration or frequent maintenance while providing continuous data logging during both ozonation reaction and storage time.

It could be considered that, when ozone inlet was performed by using the Drechsel bottle with Drechsel bottle heads in the absence or in the presence of sintered filter disk, a large vortex is always formed, resulting in significant mixing of the liquid phase and high ozone dispersion and reactivity, leading to a high efficiency of the various systems.

In conclusion, these results suggest that physico-chemical properties can provide valuable information about ~~of~~the ozonation level of SO and its potential applicability in dermatological infective pathologies. The evaluation of the oxygenated compound derived by ozonation processes is necessary in order to deepen the mechanism of action of these products. The future objectives will be the ongoing preclinic studies of ~~their~~ both their antimicrobial properties and in vivo assessment of ~~non-toxicity/allergenicity~~nontoxicity/allergenicity as well as the therapeutic activity of ozonated SO at different concentrations in a variety of chronic skin ulcers of patients with peripheral obstructive arterial diseases.

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Fig. 1 ~~FT-IR~~FT–IR spectroscopy of the samples after exposure to progressively increasing ozone amounts

Fig. 2 ^{1}H NMR spectra: neat sesame oil (before ozone treatment) and ozonated sesame oil (after ozone treatment, ~~90 min)~~90 min)

Fig. 3 Peroxide values at the different conditions adopted (see text for explanation)

Fig. 4 ~~FT-IR~~FT–IR spectroscopy of titration samples (SO, SO30 and SO60): a ~~—Control;~~ control, **b** ~~–~~ titration in agreement with the European Pharmacopoeia ~~monograph;~~monograph, **c** ~~—Reflux~~ reflux time ~~10 min;~~10 min, **d** ~~—Reflux~~ reflux time ~~60 min~~60 min

Fig. 5 Viscosity measurements of the samples after exposure to progressively increasing ozone amounts: open circle ~~(◯),~~ and solid circle ~~(●)~~ refer to 35 °C and room temperature, respectively