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Characterization of ozonated vegetable oils by spectroscopic and chromatographic methods

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Abstract

In this work the effect of ozonation on olive oil, soybean oil, oleic-, linoleic- and linolenic acid was studied. The effects of ozonation time on the oils and acids were analyzed by ^{1}H, ^{13}C NMR. Further, the peroxide- and acid values, the viscosity and the molar mass were determined for pure and ozonated oils.

The fatty chains in both ozonated oils showed a gradual decrease of unsaturation with the gradual increase of ozonation time. Reaction products were identified according to Criegee mechanism. The major product in the early stage of the reaction was ozonide. The disappearance of unsaturation and formation of ozonide was almost equal. Ozonation increased the peroxide and acid values for both oils, the increase being higher for soybean oil. After long ozonation times higher molar mass species, as well as low molar mass species were observed. These are interpreted as oligomeric ozonides and cross-ozonides, respectively.

Keywords: Ozonated soybean oil; Ozonated olive oil; Ozonated unsaturated fatty acids; NMR; GPC; Criegee mechanism

1. Introduction

The unsaturated triglycerides in vegetable oils give the oil many favorable properties. The double bonds are frequently also used for chemical modification of the oil, a typical example of which is hydrogenation. Ozonation of unsaturated triglycerides is another reaction that has gained much attention recently and ozonated vegetable oils have been proposed to be used in a variety of applications, e.g. as modifiers in biodiesel fuel (Soriano and Migo, 2005, 2006; Matsumura, 2002) and cutting fluid emulsions (John et al., 2004). Further, ozonated vegetable oils have been attributed antibacterial and fungicidal effects with applications in, e.g. food-, cosmetic- and pharmaceutical industry (Sechi et al., 2001; Geweely, 2006; Rodríguez et al., 2007; Díaz et al., 2006a,b; Guzel-Seydim et al., 2004; Ruiz et al., 2006; Valacchi et al., 2005; Bocci, 2006). The therapeutic use of ozonated vegetable oil is, however, still a rather controversial subject: although ozone and ozonated vegetable oils have been attributed many favorable therapeutic effects, their use has not been widely accepted in orthodox medicine (Bocci, 2006).

Several methods are available for characterization of ozonated vegetable oils, e.g. ^{1}H NMR, ^{13}C NMR, FT-IR, determination of peroxide- and acidity values, viscosity measurements, GPC, GC, GC-MS, etc. The reaction of ozone with vegetable oils produces several oxygenated compounds such as hydroperoxides, ozonides, aldehydes, peroxides, di- and polyperoxides (John et al., 2004; Díaz et al., 2005, 2006a; Soriano et al., 2003a,b). The reaction of ozone with unsaturated fatty acid ester in triglycerides is well described by the Criegee mechanism (Criegee, 1975; Bailey, 1978).

In this work olive and soybean oil were ozonated for different periods of time and analyzed. Further, oleic, linoleic and linolenic acid, the esters of which are present in the triglycerides in said oils, were similarly ozonated for different periods of time and analyzed. The effects of ozonation and ozone absorption on the chemical composition of the oils and fatty acids were studied

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by ^{1}H and ^{13}C NMR. Further, the viscosities, molar masses, peroxide and acid values were determined for the pure and ozonated oils.

2. Experimental

2.1. Solvents and reagents

Oleic acid (98%), linoleic acid (99%), linolenic acid (70%), soybean oil were obtained from Sigma–Aldrich. Olive oil and acetic acid (99.5%) was obtained from Fluka, potassium iodide (99.5%) from FF-Chemicals Oy Ab and ethanol from Altia Corporation. Chloroform (99.5%), sodium thiosulfate, starch, phenolphthalein, sodium hydroxide and sulfuric acid (96%) were obtained from J.T. Baker. Air (dry and oil free) was obtained from Woikoski Oy Ab. The chemicals were used without further purification.

2.2. Ozonation procedure

Ozone was produced by flowing air at a constant flow-rate of 5 l/min through a Gebr. Hermann Köln ozone generator. The voltage was set to 150 V for all experiments. The generated ozone/air mixture was led to a reaction vessel where ozone was bubbled through the oils or the fatty acids, respectively. The off-gas containing non-reacted ozone was led to an absorption vessel containing 200 ml acidic water and potassium iodide. The amount of ozone absorbed in the absorption vessel was determined by iodometric titration.

Five milliliters of each fatty acid was first ozonated for 5 min after which NMR spectra of each fatty acid were recorded. After the first analysis the fatty acids were further ozonated so that the total ozonation time was 120 min. The olive and soybean oils were each ozonated in batches of 200 ml for 2, 7 and 20 h, after which the respective batches were analyzed.

2.3. Analysis of ozone absorption

The amount of ozone consumed by the oils/fatty acids were determined by iodometric titration of the ozone absorbed in the absorption vessel. Ozone reacted with iodide to form free iodine:

$$O_3 + 2I^- + H_2O \rightarrow 2OH^- + O_2 + I_2 \quad (1)$$

The formed iodine was then titrated with sodium thiosulfate

$$I_2 + 2S_2O_3{}^{2-} \rightarrow 2I^- + S_4O_6{}^{2-} \quad (2)$$

with starch as indicator. The equivalence point was indicated as a color change from purple to colorless.

Thus, the ratio of the amount of ozone to the amount of thiosulfate for total reaction was

$$n(O_3) : n(S_2O_3{}^{2-}) = 1 : 2 \quad (3)$$

The rate of ozone production was determined by absorbing ozone directly into acidic potassium iodide solution in the absorption vessel in a blank experiment. The amount of ozone produced, $n_{PROD}(O_3)$, was thus given by

$$n_{PROD}(O_3) = 0.5 V_{BLANK}(S_2O_3{}^{2-}) c(S_2O_3{}^{2-}) \quad (4)$$

where $V_{BLANK}(S_2O_3{}^{2-})$ is the titrated volume thiosulfate solution in the blank experiment and $c(S_2O_3{}^{2-})$ is the concentration of the thiosulfate solution.

The amount of ozone consumed by the oils or fatty acid, $n_{CONSUMED}(O_3)$, is given by the difference of the amount of ozone produced during the respective ozonation procedure and the non-reacted ozone that was absorbed

$$n_{CONSUMED}(O_3) = 0.5[V_{BLANK}(S_2O_3{}^{2-}) - V_{OZONATION}(S_2O_3{}^{2-})] c(S_2O_3{}^{2-}) \quad (5)$$

where $V_{OZONATION}(S_2O_3{}^{2-})$ is the volume thiosulfate solution used to titrate the non-reacted ozone from the ozonation of the oils/fatty acids (Eaton et al., 1998).

2.4. Peroxide value (PV)

Peroxide value is the number that expresses, in milliequivalents of active oxygen, the quantity of peroxide contained in 1000 g of the substance (British Pharmacopoeia, 2000a). The PV of each sample was determined using the American Oil Chemists' Society (AOCS) official method. The chemical reactions of the AOCS official PV are presented as follows

$$2KI + 2CH_3COOH \rightarrow 2HI + 2CH_3COO^-K^+ \quad (6)$$

$$R^{\bullet}OO^{\bullet}H + 2HI \rightarrow ROH + H_2O + I_2 \quad (7)$$

Peroxides ($R^{\bullet}OO^{\bullet}H$) are reacted with potassium iodide in the presence of acetic acid, and the liberated iodine is titrated with sodium thiosulfate solution according to reaction (2). AOCS peroxide value is calculated by employing the following formula (AOCS, 1998):

$$PV = Vc\frac{1000}{w} \quad (8)$$

where PV is the peroxide value (meq/kg fat), V the volume $Na_2S_2O_3$ used, c the concentration of $Na_2S_2O_3$, w is the mass of fat.

2.5. Acid value (AV)

Acid value is the number of mg of sodium hydroxide required to neutralize the free acids in 1.0 g of the substance (British Pharmacopoeia, 2000b).

Thus, the acid value indicates the degree to which the triglycerides in the oil have broken down to release free fatty acids.

AV of the oil samples was determined by dissolving 2 g of each oil in 2 ml of neutral alcohol in a measuring flask to obtain a solution, which was titrated with sodium hydroxide in triplicate, using phenolphthalein as indicator. The acid value was calculated from

$$AV = Mc\frac{V}{w} \quad (9)$$

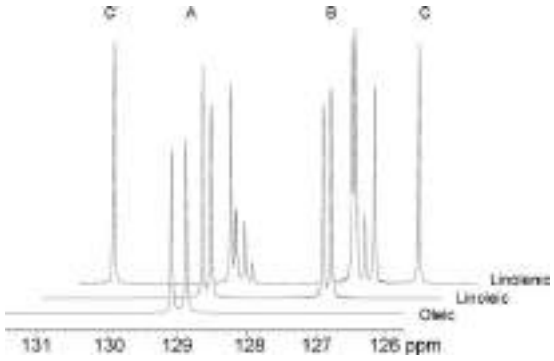

Fig. 1. Double bond peaks in the ^{13}C spectrum for pure oleic acid, linoleic acid, and linolenic acid. Peaks for each characteristic double bond carbon are assigned the letters A for the peak found in all acids, B for the peak found in both linoleic and linolenic acid, and C and C' for the peaks found only in linolenic acid.

where AV is the acid value (mg NaOH/g fat), M the molar mass of NaOH, c the concentration NaOH, V the volume of NaOH used, w is the mass of oil sample.

2.6. NMR analysis

High resolution NMR spectroscopy was used to identify the oil compositions as a function of ozonation time. A Bruker Avance spectrometer operating at 600 MHz was used. The ^{1}H NMR spectra were obtained at 9.6 kHz spectral width, 30° pulse width (3 μs), 8 scans and 32 kb of memory were used to obtain the spectra. ^{13}C spectra were recorded at 238.3 ppm spectral width, relaxation delay 2 s, a total of 500 scans was collected for each sample with a 30° excitation pulse.

2.6.1. Calculation of composition

Fig. 1 reveals the alkene carbon peaks of the ^{13}C spectra of the three reference fatty acids. Oleic acid possesses one double bond which gives two distinct peaks at 129 ppm (peaks A). Linoleic acid on the other hand has two separate double bonds and therefore has additionally two distinct peaks at 127.5 ppm (peaks B). Finally, linolenic acid has three separate double bonds giving additional peaks at 126.5 ppm (peaks C) and 131 ppm (peaks C′). These peaks were used to identify the alkene composition of the ester chains in the triglycerides. As a basis for such a calculation the integral ratios of the peaks for the pure acids has to be found. In linolenic acid the ratio of peak C to peak B is $R_{CB} = I_C/I_B = 0.277$. Compared to peak A the ratio of peak C is $R_{CA} = I_C/I_A = 0.562$. In linoleic acid the two pairs of distinct peaks have the same intensity. When the ^{13}C spectra of the triglycerides are analyzed by integration of the peaks it is easy to find the double bond composition in the ester chains by first finding the fraction with three double bonds, then the fraction with two double bonds, and finally the fraction with one double bond.

2.7. GPC-measurements

Molar masses were determined with a Shimadzu GPC instrument with a Sedex 85 Sedere LT-ELSD dectector. The instrument had one guard column (Jordi RP-DVB SM-500, 7.5 mm × 4.6 mm) and two analytical columns, both 30 cm × 7.8 mm, with Jordi Gel DVB 500A and TSG-gel G300, respectively. HPLC-grade tetrahydrofuran was used as carrier solvent at a flowrate of 1.0 ml/min. The samples were dissolved in HPLC-grade tetrahydrofuran to a concentration of 0.5 mg /ml. C18 triglycerides, C17-cholesterol-ester and C18 fatty-acids were used for calibration. The results were analyzed with Shimadzu Class-VP (v. 6.12 SP5) software. The molar masses were calculated from the retention times and the relative amounts from the relative peak areas.

2.8. Density and viscosity measurements

The density measurements were carried out with an Anton-Paar DSA5000 vibrating tube densitometer and the viscosity was measured with Ostwald capillary viscometers at 25–40 °C.

3. Results and discussion

Fig. 2a and b shows the ^{1}H and ^{13}C NMR spectra of non-ozonated and ozonated linolenic acid. Tables 1a and 1b summarize the assignments of all pertinent peaks seen in the

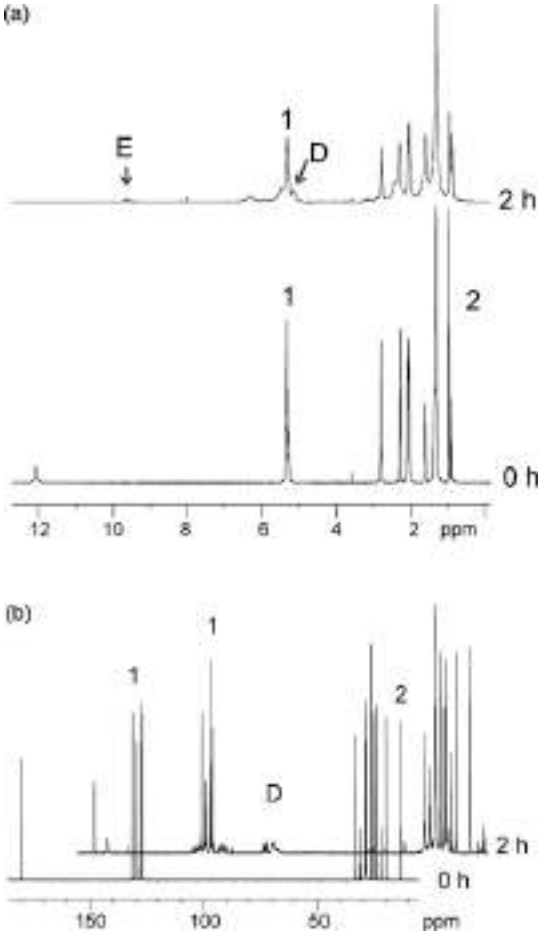

Fig. 2. (a) Full ^{1}H-spectrum of linolenic acid for the pure acid and after 2 h ozonation. (b) Full ^{13}C-spectrum of linolenic acid for the pure acid and after 2 h ozonation.

Table 1a
^{1}H NMR and ^{13}C NMR assignments of functional groups

13 C Shift [ppm]	1H Shift [ppm]	Functional group	Assignments	Signal
179		Carboxylic acid		
128–130	5.3	CH=CH	All unsaturated fatty acids	1
	5.2	CH–OCOR	Triglycerides	
61.2; 68.33	4.22; 4.42	CH_2–OCOR	Triglycerides	
24.9	2.8	$CH{=}CHCH_2CH{=}CH$	Linolenyl and linoleyl chains	
33.19	2.32	CH_2–COOH	All acyl chains	
26.5	2.04	$CH_2CH{=}CH$	All unsaturated acyl chains	
22.1	1.63	CH_2–CH_2COOH	All acyl chains	
29–31	1.3–1.5	$(CH_2)_n$	All acyl chains	
33.19	0.95	$CH{=}CHCH_2CH_3$	Linolenyl chains	
13.35	0.89	CH_3	All acyl chains except linolenyl	2

studied NMR spectra (Soriano et al., 2003b; Díaz et al., 2003; Ledea et al., 1997). The integrated intensity of the signals was normalized to the terminal methyl group of the acid moiety. This functionality was chosen because it was not affected by ozonation.

A new signal at 5.1 ppm was found in the ^{1}H NMR spectra of ozonated samples. It was assigned to the ring proton of 1,2,4-trioxolane (Soriano et al., 2003a; Díaz et al., 2005) and at 104.5 ppm in ^{13}C NMR spectra, the ring carbon in the same structure. The formation of 1,2,4-trioxolane with linoleic and linolenic acid with several double bonds in the chain shifted the signal at 5.3–5.5 ppm. Ozonated samples exhibited also peaks at 9.6 and 2.5 ppm in ^{1}H NMR corresponding to the aldehydic proton. In ^{13}C NMR, the carbonyl carbon resonated at 200 and 43 ppm, respectively.

The same peaks as for the pure acids are observed also for the oils and in addition peaks originating from the triglycerides. The effect of the ozonation time for the oils is shown in Fig. 3a and b.

The reaction with ozone is suggested to follow the Criegee mechanism. The decrease of the signal at 5.3 ppm in ^{1}H NMR and at 128–130 ppm in ^{13}C NMR is interpreted as the consumption of carbon–carbon bond. This is accompanied by the formation of 1,2,4-trioxolane. The disappearance of unsaturation and the formation of ozonide were almost equal. Further the ozonide to aldehyde ratio, regardless of ozonation time, was always above 90%. This together indicates that the major product in the early stage of the reaction is ozonide. After 2 h ozonation a new peak at 100 ppm in ^{13}C NMR spectra occurs, which is interpreted as formation of small oligomers. This is in good agreement with GPC results (Fig. 4a and b), which show that new molar mass size classes occur, corresponding to dimers respectively trimers. After 20 h ozonation compounds with even higher molar masses were found. A small fraction of compounds with lower molar masses appears after 7 h and after 20 h of ozonation this fraction becomes a bit larger. This is again in good agreement with the Criegee mechanism: cross-ozonation would produce species of low molar mass as well as species of higher molar mass. This is in agreement with the relatively low peroxide and acid values, indicating that only a small part of the reaction results in acid and oligomeric peroxide fragments.

Fig. 5 shows the consumption of the carbon–carbon bond and in Table 2 the concentration of the non-reacted ozone absorbed given as a function of the ozonation time for the studied acids and oils. The ozonation time for complete consumption of carbon–carbon bonds varies from 2.26 h for oleic acid, 2.65 h for linoleic acid and 2.95 h for linolenic acid. The corresponding times for the oils were 20 h. The same trend was obtained by the titration method (Table 2). The difference between the pure acids and the oils could partly be explained by the difference in the volumes of the ozonated samples, but some kind of steric hindrance of the reaction in the oils can not be ruled out.

According to the method described in the experimental section, the composition of the oils is calculated from NMR spectra and is given as a function of the ozonation time in Table 3.

Table 1b
^{1}H NMR and ^{13}C NMR assignments of new signals found in ozonated samples

13 C Shift [ppm]	1H Shift [ppm]	Functional group	Signal
104.5	5.1	1,2,4-Trioxolane	D
202	9.6	Aldehyde	E
43	2.50	α-Methylene group	

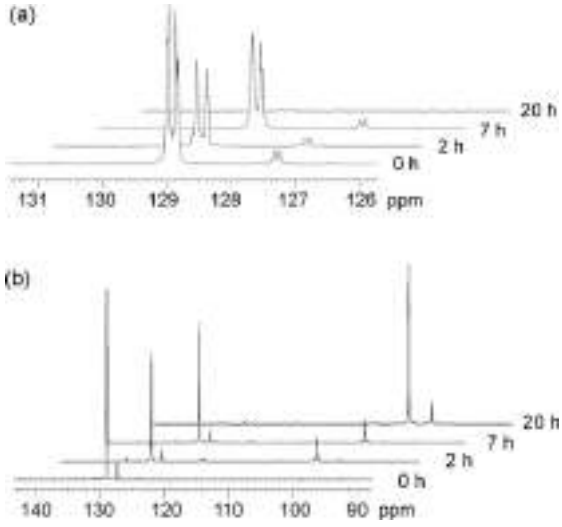

Fig. 3. (a) ^{13}C signals from the double bonded carbons in olive oil for different ozonation times. (b) ^{13}C signals from the double bonded carbons (128–130 ppm) and 1,2,4-trioxolane (104.5 ppm) in olive oil for different ozonation times.

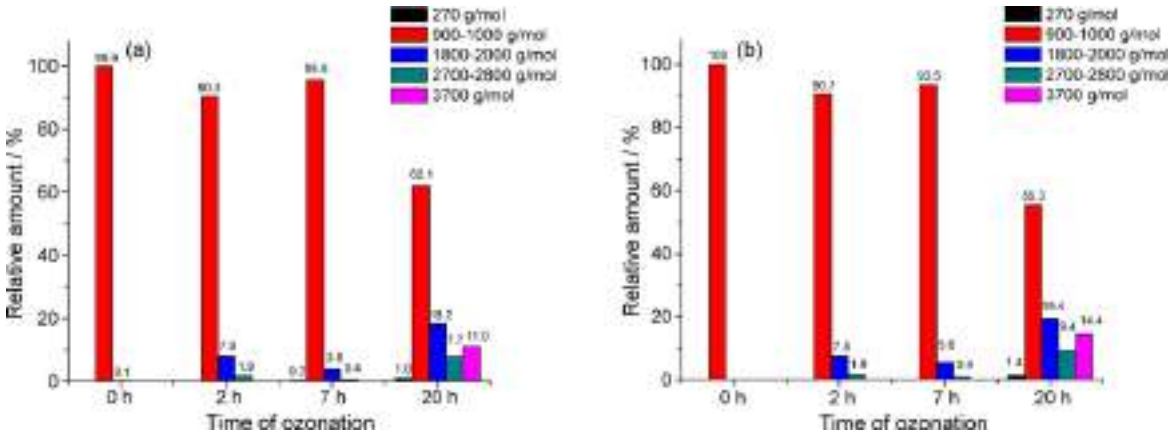

Fig. 4. (a) Relative amounts of component with different molar masses in pure olive oil and olive oil that have been ozonated for 2 h, 7 h and 20 h. (b) Relative amounts of component with different molar masses in pure soybean oil and soybean oil that have been ozonated for 2 h, 7 h and 20 h.

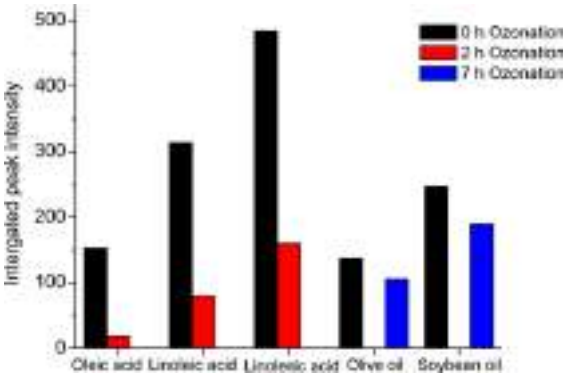

Fig. 5. The integrated intensity of the peak for the acids and the oils at 128–130 ppm in ^{13}C spectra as a function of the ozonation time.

The triglyceride fatty chain composition in both ozonated oils showed a gradual decrease in the amount of unsaturated fatty chain with the gradual increase of ozonation time.

Table 4 shows peroxide and acid values for the untreated and ozonated oils studied. The values for non-ozonated oils are in agreement with other published data for corresponding systems. The peroxide values for the ozonated oils are however not as high as reported by others (Díaz et al., 2006a). The lower peroxide values can partly be explained by the fact that no protic solvents were used in this work. An increase in peroxide and acid values was observed in both oils, but it was higher in ozonated soybean oil. These results can be explained by the higher proportion of unsaturated fatty chains in soybean oil than in olive oil.

The viscosity is usually used to characterize the fluid texture. As is seen in Fig. 6, in vegetable oils, viscosity decreases with increasing unsaturation of triglyceride fatty chains. The viscosity decreases with temperature increase. This is due to a higher thermal movement among molecules, reducing intermolecular forces, making flow among them easier. The difference in unsaturation is also reflected in the temperature dependency of the viscosity for untreated oils, but is almost the same for both ozonated oils. The increased viscosity for ozonated oils indicated that the double bonds in the oil molecules reacted with ozone form species of higher molar masses, as also seen in the molar mass measurements.

Table 2
Concentration of non-reacted ozone absorbed

Duration of ozonation	Concentration of non-reacted ozone absorbed [mmol O_3/ml]			Duration of ozonation	Concentration of non-reacted ozone absorbed [mmol O_3/ml]	
	Oleic acid	Linoleic acid	Linolenic acid		Olive oil	Soybean oil
5 min	0.088	0.064	0.036	2 h	0.00085	0.00085
2 h	0.032	0.074	0.098			

Table 3
Percentage composition of unsaturated fatty acids in both oils

Unsaturated fatty acids	Olive oil	Ozonized olive oil after 7 h	Soybean oil	Ozonized soybean oil after 7 h
C18:1 Oleic acid	78	60.6	24	18
C18:2 Linoleic acid	10	7.9	54	39.4
C18:3 Linolenic acid			7	4.48
1,2,4-Trioxolane	0	16	0	13.5
Aldehyde	0	0	0	0

Table 4
Peroxide values and acid values for pure and oils ozonated for 7 h

	Peroxide value meq O_2^{2-}/kg fat		Acid value mg NaOH/g fat	
	Untreated oil means ± S.D.	7 h ozonated oil means ± S.D.	Untreated oil means ± S.D.	7 h ozonated oil means ± S.D.
Olive oil	8 ± 0.2	28 ± 0.1	0.3 ± 0.02	1.7 ± 0.03
Soybean oil	3.2 ± 0.1	37 ± 0.1	0.5 ± 0.03	1.9 ± 0.1

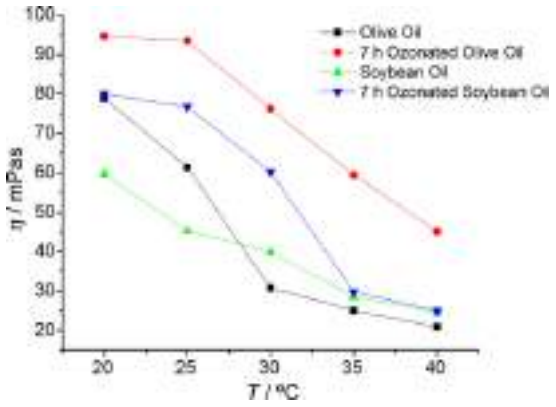

Fig. 6. The dynamic viscosity of olive oil, soybean oil, 7 h ozonated olive oil and 7 h ozonated soybean oil at different temperatures.

4. Conclusions

Ozonolysis of fatty acids, olive oil and soybean oil was suggested to follow the Criegee mechanism. The reaction of ozone with vegetable oils occurs almost exclusively with carbon-carbon double bonds present in unsaturated fatty chains. The disappearance of unsaturation and the formation of ozonide were almost equal. The ozonide to aldehyde ratio was always above 90%, which indicates that the major product in the early stage of the reaction was ozonide. The ozonation time for complete consumption of the double bonds was ten times longer for the oils than for the pure fatty acids.

Pure, non-ozonated olive and soybean oil consists almost entirely of molecules with a molar mass 900–1000 g mol^{-1}, which is in agreement with the composition of triglycerides in the oils. Ozonating the oils for 2 h decreases the amount of these components and new molar mass size classes corresponding to ozonide dimers and trimers occur. After 20 h of ozonation compounds with even higher molar masses were found. The presence of species of low molecular weight species after long ozonation times indicated that cross-ozonides were formed.

An increase in peroxide and acid values was observed in both oils, but it was higher in ozonated soybean oil. The relatively low peroxide and acid values together with the small fraction of compounds with low molar masses observed in the chromatography experiments indicate that the scission of ozonide was small.

The viscosity measurements showed that viscosity is a function of molecules' dimension and orientation. The decrease in the degree of unsaturation and the increase in molar mass both contribute to the increase in viscosity of the ozonated oils.

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References

AOCS, 1998. Peroxide value. AOCS official method. In: Firestone, D. (Ed.), Official Methods and Recommended Practices of AOCS, 5th ed. American Oil Chemists' Society, Champaign, IL, pp. 8–87.

Bailey, P.S., 1978. Ozonation in Organic Chemistry, vol. 1. Academic Press, New York, pp. 25–37.

Bocci, A.V., 2006. Scientific and medical aspects of ozone therapy. State of the art. Arch. Med. Res. 37, 425–435.

British Pharmacopoeia, 2000a. Appendix XF, IA, IB. Peroxide value.

British Pharmacopoeia, 2000b. Appendix XF, IA, IB. Acid value.

Criegee, R., 1975. Mechanismus der Ozonolyse. Angew. Chem. 87 (21), 765–771.

Díaz, M.F., Hernández, F., Ledea, O., Gavín Sazatornil, J.A., Moleiro, J., 2003. ^{1}H NMR study of methyl linoleate ozonation. Ozone Sci. Eng. 25, 121–126.

Díaz, M.F., Gavín Sazatornil, J.A., Ledea, O., Hernández, F., Alaiz, M., Garcés, R., 2005. Spectroscopic characterization of ozonated sunflower oil. Ozone Sci. Eng. 27, 247–253.

Díaz, M.F., Hernández, R., Martínez, G., Vidal, G., Gómez, M., Fernández, H., Garcés, R., 2006a. Comparative study of olive oil and ozonized sunflower oil. J. Braz. Chem. Soc. 17 (2), 403–407.

Díaz, M.F., Gavín, J.A., Gómez, M., Curtielles, V., Hernández, F., 2006b. Study of ozonated sunflower oil using ^{1}H NMR and microbiological analysis. Ozone Sci. Eng. 28, 59–63.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., 1998. Section 4500-Cl B, Iodometric method I Standard Methods for the Examination of Water and Wastewater, 20th ed. American Public Health Association, Washington, DC.

Geweely, N.S.I., 2006. Antifungal activity of ozonized olive oil (Oleozone). Int. J. Agric. Biol. 8 (5), 670–675.

Guzel-Seydim, Z.B., Greene, A.K., Seydim, A.C., 2004. Use of ozone in food industry. Lebensm. Wiss. Technol. 37, 453–460.

John, J., Bhattacharya, M., Raynor, P., 2004. Emulsions containing vegetable oils for cutting fluid application. Colloids Surf. A 237, 141–150.

Ledea, O., Díaz, M., Moleiro, J., 1997. Comparative ^{1}H NMR study of oleic acid and methyl oleate ozonation in a bubbling reactor. In: Proceedings of the XIII Ozone World Congress, Kyoto, Japan, pp. 289–294, B1-1-1.

Matsumura, M., 2002. Production of Diesel Engine Fuel by Ozonation of Sunflower Seed Oil. Patent registration # 2002GP010.

Rodríguez, Z.B.Z., Álvarez, R.G., Guanche, D., Merino, N., Rosales, F.H., Cepero, S.M., González, Y.A., Schulz, S., 2007. Antioxidant mechanism is inlvolved in gastroprotective effects of ozonized sunflower oil in ethanol-induced ulcers in rats. Mediators Inflamm 2007, 6 pages.

Ruiz, H., Ramos, L., Artiles, A., León, O., Benítez, I., 2006. Acute ulceronecrontizing gingivitis (aung). An alternative of treatment with ozonized oil. Pharmacology 3, 772–777, online.

Sechi, L.A., Lezcano, I., Nunez, N., Espim, M., Duprè, I., Pinna, A., Molicotti, P., Fadda, G., Zanetti, S., 2001. Antibacterial activity of ozonated sunflower oil (Oleozon). J. Appl. Microbiol. 90, 279–284.

Soriano Jr., N.U., Migo, V., 2005. Crystallization behavior of neat biodiesel and biodiesel treated with ozonized vegetable oil. Eur. J. Lipid Sci. Technol. 107, 689–696.

Soriano Jr., N.U., Migo, V., 2006. Ozonized vegetable oil as a pour point depressant for neat biodiesel. Fuel 85, 25–31.

Soriano Jr., N.U., Migo, V.P., Matsumura, M., 2003a. Ozonation of sunflower oil: spectroscopic monitoring of the degree of unsaturation. J. Am. Oil Chem. Soc. 80 (10), 997–1001.

Soriano Jr., N.U., Migo, V.P., Matsumura, M., 2003b. Functional group analysis of sunflower oil methyl esters by FT-IR and NMR. Chem. Phys. Lipids 126, 133–140.

Valacchi, G., Fortino, V., Bocci, V., 2005. The dual action of ozone on the skin. Brit. J. Dermatol. 153, 1096–1100.