

## Study on the stoichiometry and reaction products of extra virgin olive oil ozonation

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The amount of consumed ozone during ozonation of olive oil has been determined on the basis of continuous measurement of ozone concentrations at the bubbling reactor outlet, under conditions of constant values of ozone concentrations at the reactor inlet. This amount has been used for evaluation of reaction stoichiometry, according to which one conditional average molecule olive oil reacts with about three molecules of ozone.

The IR-spectroscopy and <sup>1</sup>H-NMR spectroscopy have been applied for identification and quantitatively characterization of basic ozonolysis products: ozonides and aldehydes. It has also been found out that *cis* and *trans* ozonides are formed and their ratio has been determined.

**Keywords:** ozone, ozonolysis, olive oil, *cis/trans* ozonides, olive oil unsaturation, <sup>1</sup>H-NMR spectroscopy

### INTRODUCTION

Recently, there has been increasing interest of cosmetic and pharmaceutical industries in the application of ozonized unsaturated triglycerides of vegetable oils [1-3]. It has been found out that the products of vegetable oils ozonolysis have antibacterial, fungicidal and antiviral properties; results of their successful utilization in dermatology for the treatment of acute cutaneous wound were reported [4-6]. At the same time their wider application is facing resistance from the orthodox medicine [7, 8]. One of the reasons for such kind of attitude is the insufficiently complete characterization of the nature and properties of the ozonolysis products. The ozonated vegetable oils have been used as modifiers in biodiesel fuel [9-11] and as additives to cutting fluid emulsions [12].

In interpreting the results of ozonolysis, the authors usually refer to the classical Criegee's scheme, according to which, when the reaction is carried out in neat substrate or in nonparticipating solvents, ozonides (1,2,4 trioxolanes) are obtained as the main product. There are few studies, in which attempts have been made to characterize all the obtained functional groups [13]. The results on the ratio between ozonides and aldehydes are contradictory [13, 14]. Although, the formation of two isomeric forms of 1,2,4 trioxolanes (*cis* and

*trans*), is well known, the structural and isomeric composition of the ozonides, obtained during ozonolysis of vegetable oils has practically not been studied yet [13, 15]. As an example of the complexity of this problem the study on methyl oleate ozonolysis can be distinguished where six isomeric ozonides (*cis* and *trans* forms of the normal type and two other types of cross-ozonides) have been identified [15].

In the present paper we monitor the ozone absorption during reaction of olive oil in neat form or in nonparticipating solutions. The ozonolysis products were identified and quantitatively determined by FT-IR and <sup>1</sup>H-NMR spectroscopy.

### EXPERIMENTAL

Ozone was prepared by passing oxygen through a 4-9 kV discharge.

The experiments have been performed in a bubbling reactor, containing 5-10 mL of olive oil or olive oil solutions in CCl<sub>4</sub>, equipped with a thermostating water jacket. An ozone-oxygen mixture with ozone concentration in the range of 1000–25000 ppm ( $4.5 \cdot 10^{-5}$ - $1.2 \cdot 10^{-3}$  mol L<sup>-1</sup>) was passed through the reactor at a flow rate of  $(1.67 \pm 0.15) \cdot 10^{-3}$  L s<sup>-1</sup>. The ozone concentrations in the gas phase at the reactor inlet ([O<sub>3</sub>]<sub>o</sub>) and outlet ([O<sub>3</sub>]<sub>u</sub>) have been measured by OZONE ANALYZER BMT 964 device.

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Infrared spectra were recorded on thin films coated onto KBr plates using Nicolet 6700 FTIR spectrometer: The spectra comprise the result of 100 scans with a resolution of  $4\text{ cm}^{-1}$ .

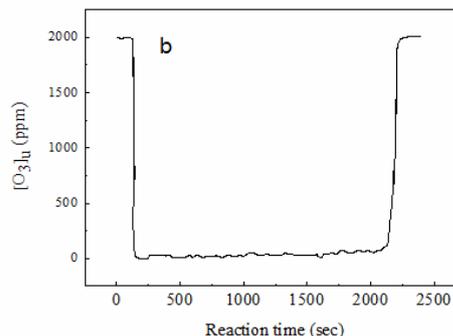
$^1\text{H-NMR}$  spectra were recorded on a Bruker Avance 600 MHz instrument under the following conditions:  $20\text{ }^\circ\text{C}$ ; digital resolution  $0.3\text{ Hz}$ ; number of scans 30. Samples were dissolved in  $\text{CDCl}_3$  containing TMS as internal standard.

## RESULTS AND DISCUSSION

### Monitoring of the ozone absorption

The kinetics of the ozone absorption has been studied by continuous monitoring of ozone concentrations at the reactor outlet ( $[\text{O}_3]_u$ ), under condition of constant values of initial ozone concentration at the reactor inlet ( $[\text{O}_3]_o$ ).

The kinetics of ozonolysis of different double bonds in solution is considerably less investigated than the corresponding mechanism [16]. Because of the high rates of ozonolysis of alkenes, whose double bonds are structurally similar to the  $\text{RHCH}=\text{CHR}_1\text{H}$  bonds of 3-glycerides in olive oil, the rate constant values, published in the literature, are determined mainly by three methods: 1)- measuring the relative rate of consumption of the investigated olefin, comparing with a control compound; 2) – determining of the  $[\text{O}_3]_u$  values and 3)- stop-flow method [17-19]. Although there are some differences, the values of the rate constants of alkenes, discussed above, are in the range of  $5 \cdot 10^4$ - $5 \cdot 10^5\text{ L mol}^{-1}\text{ s}^{-1}$ . Based on the features of olefins ozonolysis, a spectrophotometric method has been developed for quantitative determination of the double bonds by means of measuring the amount of absorbed ozone [17, 20]. This method could be competitive to the iodine value method determination and it has a very high sensitivity due to the great values of the extinction coefficient of ozone absorption [21]. The essence of the method is illustrated in Fig. 1, where the dependence of  $[\text{O}_3]_u$  on the time of ozonation of olive oil solution in  $\text{CCl}_4$  is shown under conditions of normal bubbling regime and  $[\text{O}_3]_o = \text{const}$ . The respective curve is characterized by two practically perpendicular sections at the beginning and at the end of the reaction, and region where  $[\text{O}_3]_u \approx 0$ . The absence of outlet ozone is due to the high values of the rate constant of ozone reaction with double  $\text{C}=\text{C}$  bonds [18]. The surface of the area integrated between the curves  $[\text{O}_3]_u = f(\tau)$  and the line  $y = [\text{O}_3]_o$  (where  $[\text{O}_3]_o = [\text{O}_3]_u$  at  $\tau = 0$ ) is proportional to the amount



**Fig. 1.** Dependence of the ozone concentration at the reactor outlet ( $[\text{O}_3]_u$ ) on the reaction time for solution of olive oil in carbon tetrachloride ( $0.1\text{ mL}$  in  $10\text{ mL CCl}_4$ ) ozonolysis.

of consumed ozone. This amount has been calculated by using the coefficient of ozone absorbance at UV-C wavelength  $254\text{ nm}$  [21] and the respective flow rate of the ozone–oxygen mixture. At appropriately selected concentrations of double bonds in the solution, the surface area of the solvent saturation with ozone becomes negligible compared to the curve area of the olive oil solution and it may be neglected. The degree of unsaturation of olive oils is usually measured by the so called “iodine value”. Its determination is based on the reaction of double bonds with halogens (iodine), resulting in the formation diiodine alkanes. The iodine value is the mass (in grams) of halogens, expressed as iodine, which is absorbed by 100 grams of olive oil. The amount of measured ozone, shown in Fig. 1, is  $3.02 \cdot 10^{-4}\text{ mol}$ . Assuming that it is equal to that of the double bonds, normalizing it to  $100\text{ g}$  of olive oil and recalculating into grams of iodine, according to the methodology for determining of the iodine value [22, 23] we obtained  $83.4\text{ g}$  of iodine, absorbed by  $100\text{ grams}$  of olive oil. Hence the iodine number of olive oil, determined on the basis of its ozonolysis in solution was  $83.4$ . This value is within the range, known from the literature to be specific for the iodine number of olive oils [24].

### Functional group analysis

A broad and strong band at  $1109\text{ cm}^{-1}$  is seen in the IR spectra of ozonated olive oil (Fig. 2), assigned to the  $\text{C-O}$  stretching vibration of ozonide [17, 25] and the band at  $1727\text{ cm}^{-1}$  is typical for  $\nu\text{ C=O}$  of aldehydes [26]. In addition to these two signals, an intensive peak at  $1379\text{ cm}^{-1}$  and a shoulder at  $975\text{ cm}^{-1}$  were also identified. It is mentioned in the literature that the *trans* isomer of

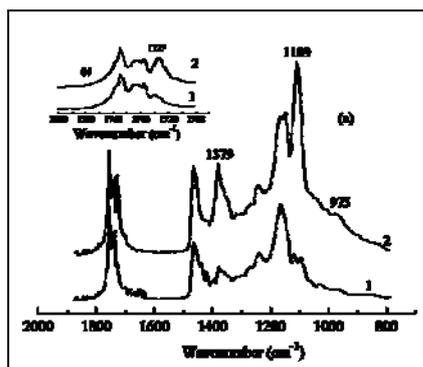


Fig. 2. Infra-red spectra of: non-ozonized (1) and ozonated (2) olive oil in: 800-2000 cm<sup>-1</sup> region (a); 1700-1800 cm<sup>-1</sup> region (b).

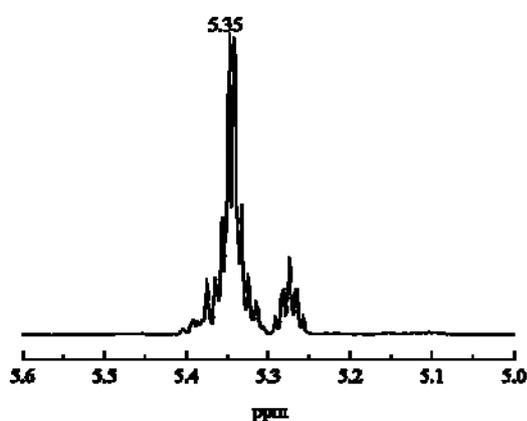


Fig. 3a. <sup>1</sup>H NMR spectra of neat olive oil in the region of 5.0-5.5 ppm.

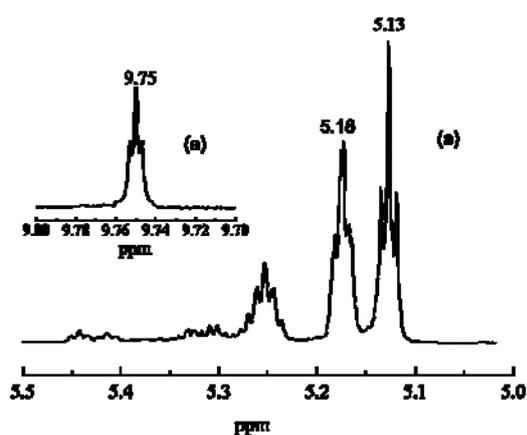


Fig. 3b. <sup>1</sup>H NMR spectra of ozonated olive oil in the region of 5.0-5.5 ppm (a) and 9.70-9.80 ppm (a').

the 1,2,4-trioxolane absorbs at about 1300 cm<sup>-1</sup>, while the *cis* isomer absorbs at about 800 cm<sup>-1</sup> [25]. According to another work, upon ozonolysis of sunflower oil methyl esters, a broad peak at 1365 cm<sup>-1</sup> appears and on this basis it is concluded that the corresponding *trans* ozonides are dominating

products [13]. According to the experimental reference data from applied IR-spectroscopy, the peak at 1379 cm<sup>-1</sup> is most likely associated with ν C-O-C [26]. The band at 975 cm<sup>-1</sup> is characteristic for the stretching vibration of ν O-O [26].

The <sup>1</sup>H-NMR spectroscopy provides much more opportunities for identification and quantitative determination of functional groups formed during ozonolysis of olive oil. Assignment of the signals of non-ozonized olive oil (Fig. 3a) is discussed in detail by Vlahov [27]. There exist two isomeric forms of 1,2,4-trioxolanes: *cis* and *trans* [25]. Their ratio is a function of the double bond stereochemistry, the steric effect of the substituent, and the conditions of ozonolysis, and it has been studied only in cases of low-molecular-weight alkenes [25, 28, 29]. The signal of the *cis* form appears at lower field intensity, compared with the *trans* one [25, 28]. The *cis/trans* ratio of the olive oil ozonides, determined from integrated intensity of signals at 5.18 and 5.13 ppm was 46:54 (Fig. 3b).

The formation of the basic ozonolysis product—the ozonide is represented in Fig. 4. The modern concepts about the mechanism of the reaction of ozone with C=C double bonds in nonparticipating solvents, in contrast to the classical Criegee's mechanism, take into account the stereochemical features of the reaction, which ultimately leads to formation of *cis* and *trans* 1,2,4 – trioxolanes [16, 25, 29].

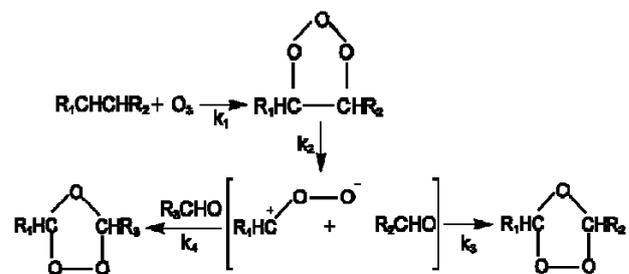


Fig. 4. Ozonation and formation of ozonides

The initial reaction is 1,3 coordinated addition of ozone to *cis(trans)* C=C double bond with formation of *cis-* or *trans-*molozonide (MO) respectively. The *cis-* or *trans-*molozonide are unstable species, and at temperatures higher than -150°C for the *cis*-isomer and -90°C for the *trans*-isomer it is decomposing via the so-called "cycloreversion" (reaction 2) [29]. It is assumed that as a result of the passing of the reaction through the envelope transition state, predominantly *anti* carbonyl oxides are obtained from *cis* isomers of MO. The degree of the isomer preference and the extent of stereoselectivity

depend on substituent steric effects, secondary orbital interactions and anomeric interactions [29]. Ozonides are formed as a result of the interaction of the corresponding carbonyl oxides with aldehydes (reaction 3). It is supposed that this interaction is of the cycloaddition type via envelope transition state according to orbital overlap arguments [29]. Thus a *syn* (or *anti*) carbonyl oxide (Fig. 5) will react with aldehyde in either endo or exo transition state to give *cis* or *trans* ozonide. This step, like the

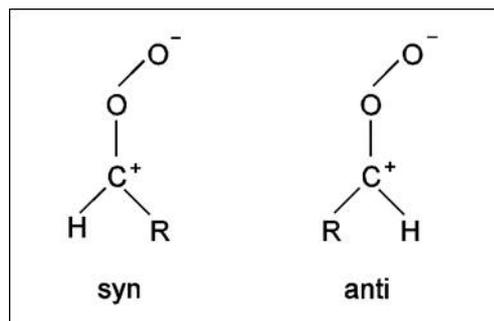


Fig. 5. Stereochemistry of carbonyl oxides intermediates.

previous one, can occur with varying degrees of stereoselectivity depending on substituent interactions, electronic effects, temperature etc [16, 25, 29]. It has been found out that the dominant part of ozonides is formed through interaction between carbonyl oxide and the corresponding carbonyl group, which both originate from the decomposition of one and the same MO, i.e., a solvent "cage effect" is occurring (Fig. 4, reaction 3) [16, 17, 25]. The yield of the so-called normal ozonides from the simple olefins is usually of the order of 70 % of the total ozonide yield [25]. Cross ozonides are products of the interaction between carbonyl oxide and aldehyde, originating from different double bonds (Fig. 4, reaction 4). It is assumed that the greater volume of the substituents hampers their formation [25, 30]. The second main type of product of the olive oil ozonolysis are the aldehydes. According to the mechanism of the ozone reaction with double bonds in solution, the aldehyde groups are being formed when the conversion of carbonyl oxide intermediates is proceeding through such routes, which are an alternative to the carbonyl oxide-aldehyde interaction [25, 30]. The nonozonide routes are discussed in detail by Anachkov [30]. The ozonide/aldehyde ratio, defined as ratio between half of integrated intensity of signals of methine protons of the *cis*- and *trans*- ozonides and

aldehyde signal at 9.75 ppm was 93.4:6.6 (mol. %). On the basis of the material balance of ozone, the stoichiometry of ozonolysis of olive oil was estimated. It was found that one molecule olive oil reacts with about three molecules of ozone.

## CONCLUSIONS

The kinetics of ozone absorption during olive oil ozonolysis has been studied by continuous monitoring of ozone concentrations at the bubbling reactor outlet, under conditions of constant values of ozone concentrations at the reactor inlet. The determined amount of ozone, consumed during the ozonolysis of the double bonds, was used as an alternative method for the quantitative determination of the degree of oil unsaturation. The basic compounds, products of the reaction: ozonides and aldehydes have been identified and quantitatively characterized by means of IR-spectroscopy and <sup>1</sup>H-NMR spectroscopy; their ratio was found out to be 93.4:6.6 (mol. %) respectively. The ratio between the *cis* and *trans* ozonides was also determined to be 46:54.

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## ИЗСЛЕДВАНЕ НА СТЕХИОМЕТРИЯТА И РЕАКЦИОННИТЕ ПРОДУКТИ ОТ ОЗОНИРАНЕТО НА ЧИСТ ЗЕХТИН

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(Резюме)

Определено е количеството на погълнатия озон по време на озониране на маслиново масло на базата на непрекъснато измерване на концентрациите на озона на изхода от барботиращ реактор, при условия на постоянни стойности на концентрацията на озона на вход на реактора. Това количество се използва за определяне на стехиометрията на реакцията, съгласно с което една условна молекула от маслиновото масло реагира с около три молекули озон. За идентифициране и количествено характеризиране на основните продукти от озонизацията: озониди и алдехиди, са приложени ИЧ и <sup>1</sup>H-NMR спектроскопии. Установено е, че в хода на реакцията се образуват цис и транс озониди, като е определено и тяхното съотношение.